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Neodymium improves the activity of ascorbate-glutathione cycle and chloroplast function of wheat seedlings under chromium stress

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Abstract

We investigated the roles of neodymium trichloride ($NdCl_3$) in regulating ascorbate-glutathione (AsA-GSH) cycle and chloroplast function of chromium (Cr)-stressed wheat seedlings. The findings showed that Cr stress markedly increased malondialdehyde (MDA) content, electrolytic leakage (EL), nonphotochemical quenching, and the activities of enzymes in AsA-GSH cycle, compared with control. However, Cr stress markedly reduced the ratios of reduced ascorbate to dehydroascorbate and reduced glutathione to oxidized glutathione, net photosynthetic rate, the contents of chlorophylls and carotenoids, maximum photochemical efficiency of PSII, photochemical quenching, and quantum efficiency of PSII photochemistry, as well as plant height, root length, and plant biomass. $NdCl_3$ plus Cr stress markedly reduced MDA content and EL and improved other indicators, compared with Cr stress alone. Meanwhile, $NdCl_3$ alone also markedly improved above indicators except MDA and EL, compared with control. Current results implied that $NdCl_3$ improved AsA-GSH cycle and chloroplast function of wheat seedlings under Cr stress.

Additional key words: chromium tolerance; Halliwell-Asada pathway; photosynthesis; *Triticum aestivum* L.

Introduction

Chromium (Cr) stress has significant inhibitory effects on plant growth and development (Qing *et al.* 2015). Cr stress usually leads to the excessive production of reactive oxygen species (ROS), which further causes oxidative stress to plants (Karuppanapandian *et al.* 2006). In fact, plants have a complicated antioxidant protection system to fight against oxidative stress. In plants, the ascorbate-glutathione (AsA-GSH) cycle is a very vital part of the antioxidant protection system (Ahmad *et al.* 2018). AsA-GSH cycle is composed of four enzymes, such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). Through the catalytic action of above four enzymes, hydrogen peroxide is scavenged and two important antioxidants, AsA and GSH, are regenerated. Thus, AsA-GSH cycle has a vital role in cleaning up H_2O_2 and maintaining the redox equilibrium of plant cells.

Rare earth elements (REEs) have obvious positive effects on plant growth, plant production, and fruit quality (Ouyang *et al.* 2003, Shan *et al.* 2017, 2018). Lanthanum (La) and cerium (Ce) are two important REEs. In plants, an increasing number of studies was focused on the roles of La and Ce in alleviating the oxidative stress induced by heavy metal stress (Wang *et al.* 2013, Dai *et al.* 2017). Neodymium (Nd) is also an important member of REEs. However, there is still no report about the roles of Nd in regulating the antioxidative responses of plants to Cr stress. Therefore, it is meaningful to clarify whether and how Nd affects the antioxidant capacity of Cr-stressed plants through AsA-GSH cycle.

Chloroplast function has a close relationship with plant growth. The results of Xue *et al.* (2018) showed that Cr stress decreased the photosynthetic activity of chloroplasts. However, there is also still no report about the effects of Nd on the photosynthetic activity of chloroplasts, especially under Cr stress. Thus, it is also meaningful to clarify whether and how Nd affects the photosynthetic activity of chloroplasts under Cr stress.

In current study, we investigated the effects of neodymium trichloride ($NdCl_3$) on the activities of enzymes in AsA-GSH cycle, the ratios of reduced ascorbate to dehydroascorbate (AsA/DHA) and reduced glutathione to oxidizided glutathione (GSH/GSSG), malondialdehyde (MDA) content and electrolytic leakage (EL), net photosynthetic rate (P_N), chlorophyll fluorescence parameters, the contents of chlorophylls (Chl) and carotenoids (Car),

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Abbreviations: APX – ascorbate peroxidase; AsA – reduced ascorbate; AsA/DHA – the ratio of reduced ascorbate to dehydroascorbate; AsA-GSH – ascorbate-glutathione; Car – carotenoids; DHAR – dehydroascorbate reductase; EL – electrolyte leakage; F_v/F_m – maximum photochemical efficiency of PSII; GR – glutathione reductase; GSH – reduced glutathione; GSH/GSSG – the ratio of reduced glutathione to oxidized glutathione; MDA – malondialdehyde; MDHAR – monodehydroascorbate reductase; P_N – net photosynthetic rate; q_N – nonphotochemical quenching; q_P – photochemical quenching; Φ_{PSII} – effective quantum yield of PSII.
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as well as plant height and biomass of Cr-stressed wheat seedlings. The aim of this study was to elucidate the effects of $NdCl_3$ on AsA-GSH cycle and chloroplast function of Cr-stressed wheat seedlings, which will provide new information for its application in enhancing Cr tolerance of wheat crops.

Materials and methods

Plant material and treatments: The seeds of wheat (*Triticum aestivum* L.) cultivar Bainong 207 were germinated and cultured in the manual climatic box (*GXM-358*, *Beijing Century Sunshine Technology Development Co*., *Ltd*., China). The temperature of day/night periods, photoperiod, and photosynthetic active radiation were set as 25/15°C, 12 h, and 500 μmol(photon) m^{-2} s^{-1}, respectively. After the second leaves were fully expanded, the roots of plants were placed in half-strength Hoagland's solution, which was replaced every second day. After the third leaves were fully expanded, plants with similar growth status were chosen for our study.

The suitable treatment concentration of $CrCl_3$ was selected from 20, 40, 80, and 120 mg($CrCl_3$) L^{-1}. After 48 h of treatment, we observed obvious wilting phenomenon for plants treated by 120 mg($CrCl_3$) L^{-1}, while no obvious wilting phenomenon was observed for plants treated by 20 and 40 mg($CrCl_3$) L^{-1}. However, there was only slight wilting phenomenon for plants treated by 80 mg($CrCl_3$) L^{-1}. Thus, 80 mg($CrCl_3$) L^{-1} was selected as the suitable concentration for Cr treatment in this study. To investigate the effect of Cr stress, the roots of wheat seedlings were treated by 80 mg($CrCl_3$) L^{-1} for 48 h. Then we investigated the effects of different concentrations of $NdCl_3$ on MDA content and plant biomass, and selected 30 μM $NdCl_3$ as the suitable concentration for the current study. Then, the roots of plants were firstly placed in 30 μM $NdCl_3$ for 12 h and then treated by Cr stress or Hoagland's solution for 48 h. Control plants were only treated by 30 μM $NdCl_3$ or Hoagland's solution. All solutions were prepared by dissolving corresponding chemicals in Hoagland's solution. After 48 h of treatment, top fully expanded leaves of wheat seedlings under different treatments were sampled and stored in liquid nitrogen and then used for measurements.

Assays of antioxidant enzymes: The extraction and activity of APX (EC 1.11.1.11) was done according to Han *et al.* (2015) and Nakano and Asada (1981). The extraction and activities of GR (EC 1.6.4.2) and DHAR (EC 1.8.5.1) were done according to Singh *et al*. (2018) and Dalton *et al.* (1986), respectively. The extraction and activity of MDHAR (EC 1.6.5.4) was done according to Li *et al.* (2015). One unit of APX activity was defined as the amount of APX catalyzing the oxidation of 1 μmol AsA per min. One unit of GR activity was defined as the reduction of 1 μmol NADPH per min. One unit of MDHAR activity was defined as the amount of enzyme that oxidizes 1 μmol NADH per min. One unit of DHAR activity was defined as the amount of enzyme that produces 1 μmol AsA per min. The specific activities of these enzymes were expressed as units mg^{-1}(protein). Protein concentration was analysed according to Bradford (1976). *TU-1810* UV-Vis spectrophotometer (*Beijing Purkinje General Instrument Co*., *Ltd*., China) was used for activity measurements.

Analysis of AsA/DHA and GSH/GSSG: AsA and DHA were analysed according to Hodges *et al.* (1996) and Zhao *et al*. (2018). The ratio of AsA/DHA was expressed by the ratio of AsA content to DHA content. GSSG and GSH were analysed according to Li *et al.* (2018). The ratio of GSH/GSSG was expressed by the ratio of GSH content to GSSG content.

Analysis of MDA content and EL: The content of MDA was analysed according to Heath and Packer (1968) and Wang *et al*. (2018). *TU-1810* UV-vis spectrophotometer (*Beijing Purkinje General Instrument Co*., *Ltd*., China) was used for MDA measurement. EL was analysed according to Anjum *et al.* (2015) and Yu *et al*. (2018). *DDSJ-308* conductivity meter (*Shanghai Leici Instrument Co*., *Ltd*., China) was used for EL measurement.

***P_N* and chlorophyll (Chl) fluorescence parameters**: After 48 h of treatment, P_N was determined by photosynthesis system (*Licor-6400*, USA) from 10:00 to 12:00 h. The top expanded leaves of different treatments were first equilibrated at a photosynthetic photon flux density of 700 μmol m^{-2} s^{-1}, 26°C, an ambient CO_2 concentration of 300 μmol mol^{-1}, and a vapour pressure difference between leaf and air of 1.0–1.2 kPa. After equilibration, steady-state gas-exchange values were recorded. Chl fluorescence parameters, such as maximum photochemical efficiency of PSII (F_v/F_m), photochemical quenching (q_P), nonphotochemical quenching (q_N), and quantum efficiency of PSII photochemistry (Φ_{PSII}) were determined by a *Yaxin-1161G* fluorometer (*Yaxin*, China) from 10:00 to 12:00 h. Measurements were performed in a closed chamber under controlled growth conditions. Minimum fluorescence (F_0) was measured under a weak modulating radiation [0.5 μmol(photon) m^{-2} s^{-1}], and maximum fluorescence (F_m) was induced by a saturating pulse of radiation [2,400 μmol(photon) m^{-2} s^{-1}].

Assays of Chl and Car: Top fully expanded leaves were used to determine the contents of Chl and Car according to Lichtenthaler and Wellburn (1983) and Song *et al*. (2016). *TU-1810* UV-Vis spectrophotometer (*Beijing Purkinje General Instrument Co*., *Ltd*., China) was used for the assays of Chl and Car.

Plant height, root length, and plant biomass: After 7 d of treatment, the ruler was used to measure root length and plant height. Fresh masses of plants under different treatments were recorded and dried in the oven for 96 h at 80°C. Then dry masses were recorded.

Measurement of Nd and Cr: After 7 d of treatment, each dry sample of roots and leaves was ground and mixed thoroughly. Fine powder (0.5 g) of each dry sample was digested in a mixture of 7 ml of HNO_3 + 1 ml of $HClO_4$

at 170°C according to Dai *et al.* (2017). Then the contents of Nd and Cr in extracts were measured by flame atomic absorbance spectrometry (*Hitachi 180-80*, Kyoto, Japan).

Table 1. Effects of different concentrations of $NdCl_3$ on malondialdehyde (MDA) content and plant biomass of wheat under Cr stress. The plants were treated as below. Control – half-strength Hoagland's solution; Cr – 80 mg L^{-1} $CrCl_3$; 10 μM Nd + Cr – 10 μM $NdCl_3$ + 80 mg L^{-1} $CrCl_3$; 30 μM Nd + Cr – 30 μM $NdCl_3$ + 80 mg L^{-1} $CrCl_3$; 50 μM Nd + Cr – 50 μM $NdCl_3$ + 80 mg L^{-1} $CrCl_3$; 100 μM Nd + Cr – 100 μM $NdCl_3$ + 80 mg L^{-1} $CrCl_3$. The plants were firstly treated by $NdCl_3$ for 12 h, and then treated by Cr stress or half-strength Hoagland's solution. After 48 h of treatment, MDA content was determined. After 7 d of treatment, plant biomass was determined. Values are means ± SD, $n = 6$. *Different lowercase letters* mean statistical difference at 5% level of significance.

Treatment	MDA [nmol g^{-1}(FM)]	Plant biomass [mg(FM) $plant^{-1}$]
Control	5.00 ± 0.43^{e}	108.00 ± 11.30^{a}
Cr	13.90 ± 1.27^{b}	80.30 ± 9.24^{c}
10 μM Nd + Cr	12.00 ± 1.11^{c}	85.60 ± 7.48^{bc}
30 μM Nd + Cr	9.70 ± 0.89^{d}	95.90 ± 9.15^{b}
50 μM Nd + Cr	12.50 ± 1.15^{c}	83.40 ± 7.99^{bc}
100 μM Nd + Cr	15.90 ± 1.81^{a}	71.00 ± 8.34^{d}

Statistical analysis: The data in tables and figures were the mean of six replications. Means were compared by one-way analysis of variance (*ANOVA*) and *Duncan*'s multiple range test at 5% level of significance. *SPSS 19.0* was used for statistical analysis.

Results

MDA content and plant biomass: To select the suitable concentration of $NdCl_3$, we investigated the effects of different $NdCl_3$ concentrations (10, 30, 50, and 100 μM $NdCl_3$) on MDA content in leaves and plant biomass of Cr-stressed wheat seedlings. Among different concentrations, 30 μM $NdCl_3$ markedly decreased MDA content in leaves and increased plant biomass of Cr-stressed wheat seedlings (Table 1). Based on above findings, we selected 30 μM $NdCl_3$ as the suitable concentration for this study.

Activities of enzymes in AsA-GSH cycle: Compared with control, Cr stress markedly improved the activities of enzymes in AsA-GSH cycle, including APX, GR, DHAR, and MDHAR (Fig. 1). Application of $NdCl_3$ plus Cr stress markedly enhanced the activities of above four enzymes, compared to Cr stress alone. Application of $NdCl_3$ plus Cr stress improved the activities of APX, GR, DHAR, and MDHAR by 34.2, 34.0, 50.8, and 35.6 %, respectively. At the same time, $NdCl_3$ alone also markedly enhanced the activities of above four enzymes, compared

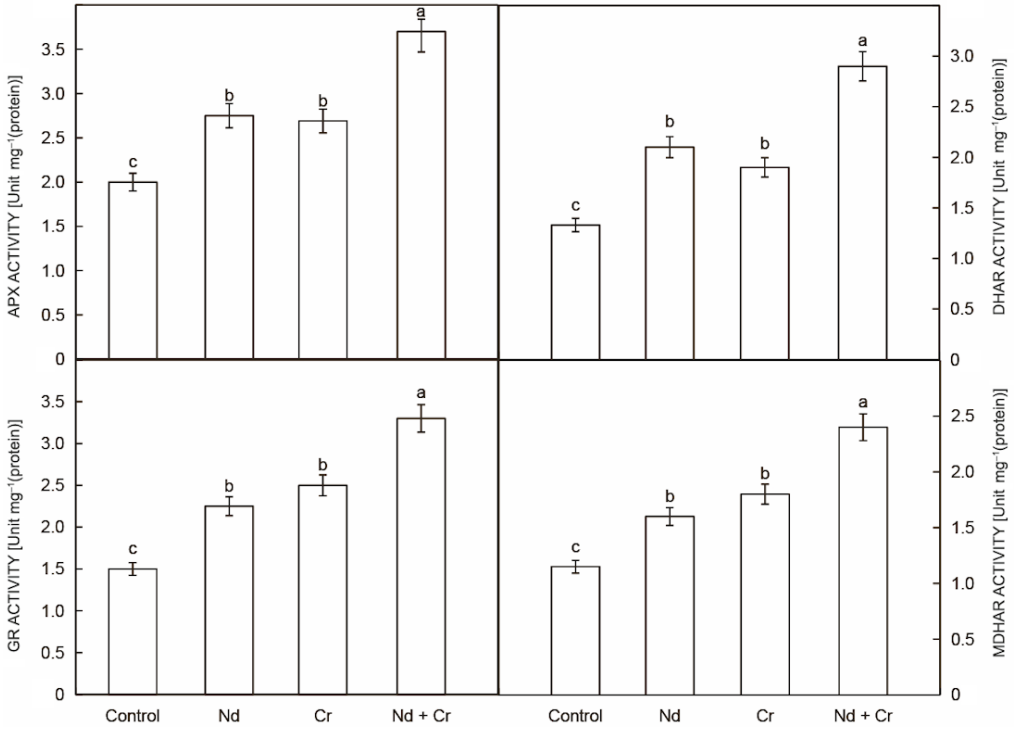

Fig. 1. Effects of Nd on the activities of ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) of wheat under Cr stress. The plants were treated as below. Control – half-strength Hoagland's solution; Nd – 30 μM $NdCl_3$; Cr – 80 mg L^{-1} $CrCl_3$; Nd + Cr – 30 μM $NdCl_3$ + 80 mg L^{-1} $CrCl_3$. The plants were firstly treated by $NdCl_3$ for 12 h, and then treated by Cr stress for 48 h. Values are means ± SD, $n = 6$. *Different lowercase letters* mean statistical difference at 5% level of significance.

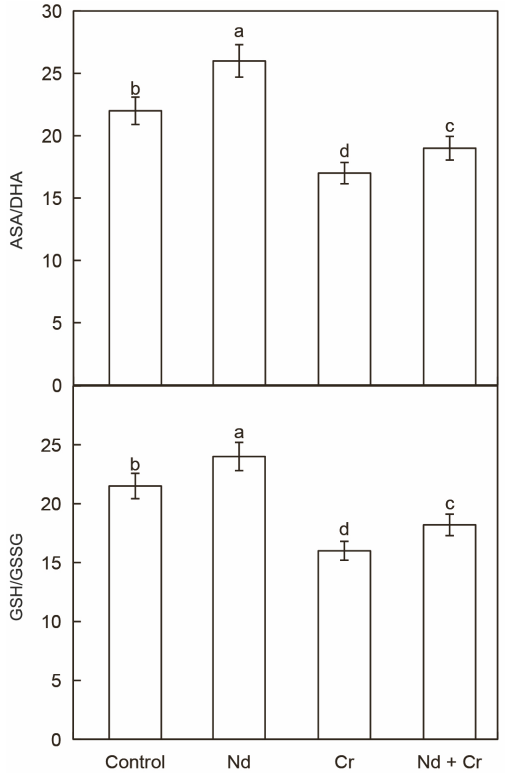

Fig. 2. Effects of Nd on the ratio of reduced ascorbate to dehydroascorbate (AsA/DHA) and the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG) of wheat under Cr stress. The plants were treated as below. Control – half-strength Hoagland's solution; Nd – 30 μM $NdCl_3$; Cr – 80 mg L^{-1} $CrCl_3$; Nd + Cr – 30 μM $NdCl_3$ + 80 mg L^{-1} $CrCl_3$. The plants were firstly treated by $NdCl_3$ for 12 h, and then treated by Cr stress for 48 h. Values are means ± SD, n = 6. *Different lowercase letters* mean statistical difference at 5% level of significance.

with control. Current results implied that $NdCl_3$ enhanced the antioxidant capacity of Cr-stressed wheat seedlings through AsA-GSH cycle.

Redox states of AsA and GSH: Cr stress markedly reduced AsA/DHA and GSH/GSSG in the leaves of wheat seedlings, compared with control (Fig. 2). Application of $NdCl_3$ plus Cr stress markedly increased above two ratios, compared to Cr stress alone. Application of $NdCl_3$ plus Cr stress increased AsA/DHA and GSH/GSSG by 13.5 and 13.9%, respectively. At the same time, $NdCl_3$ alone also markedly improved above two ratios, compared with control. Combined with the effects of $NdCl_3$ on AsA-GSH cycle, our present results suggested that $NdCl_3$ could modulate the redox states of AsA and GSH *via* AsA-GSH cycle under Cr stress.

P_N, Chl fluorescence parameters, and the contents of Chl and Car: Compared with control, Cr stress markedly decreased P_N, F_v/F_m, q_P, Φ_{PSII}, and the contents of Chl and Car, but markedly increased q_N in leaves (Table 2). Application of $NdCl_3$ plus Cr stress markedly improved above indicators, compared with Cr stress alone. Application of $NdCl_3$ plus Cr stress improved F_v/F_m, q_P, q_N, Φ_{PSII}, P_N, and the contents of Chl and Car by 22.8, 23.5, 18.2, 20.0, 23.4, 14.8, and 26.3%, respectively. At the same time, $NdCl_3$ alone also markedly improved above indicators, compared with control. Combined with the effects of $NdCl_3$ on AsA-GSH cycle, the present findings suggested that $NdCl_3$ improved the function of PSII by enhancing AsA-GSH cycle under Cr stress.

Nd and Cr content: Cr stress markedly enhanced the contents of Cr in roots and leaves, in comparison with control (Table 3). Application of $NdCl_3$ plus Cr stress markedly reduced the contents of Cr in roots and leaves, in comparison with Cr stress alone. Application of $NdCl_3$ plus Cr stress reduced the contents of Cr in roots and leaves by 32.2 and 33.7%, respectively. Meanwhile, $NdCl_3$ alone markedly increased the contents of Nd in roots and leaves, in comparison with control. Application of $NdCl_3$ plus Cr stress also markedly improved the contents of Nd in roots and leaves, compared with Cr stress alone. These findings demonstrated that $NdCl_3$ had an alleviating effect on Cr toxicity by reducing the absorption of Cr by the roots and leaves of wheat crops.

MDA content, root length, plant height, and biomass: In comparison with control, Cr stress markedly increased EL and MDA content, and decreased root length, plant height, and biomass (Table 4). In comparison with Cr stress alone, application of $NdCl_3$ plus Cr stress markedly reduced EL and MDA content, and improved root length, plant height, and biomass. Application of $NdCl_3$ plus Cr stress reduced EL and MDA content by 31.5 and 32.3%, respectively. After 7 d of treatment, application of $NdCl_3$ plus Cr stress enhanced root length, plant height, and biomass by 23.2, 18.3, and 17.6%, respectively. At the same time, $NdCl_3$ alone also markedly decreased EL and MDA content, and improved root length, plant height, and biomass, in comparison with control. These findings demonstrated that $NdCl_3$ had an alleviating effect on Cr toxicity.

Discussion

Many studies showed that heavy metal stress caused oxidative stress to plants, including Cr stress (Tan *et al.* 2014, Zhang *et al.* 2014, Mahmud *et al.* 2017). Our present study also showed that Cr stress markedly increased EL and MDA content in leaves. Thus, our findings indicated that Cr stress could also cause oxidative stress to wheat plants.

Karuppanapandian *et al.* (2006) reported that Cr stress had obvious effects on AsA-GSH cycle in green gram. Farid *et al.* (2018) showed that Cr stress upregulated APX activity in sunflower. Kabir (2016) reported that Cr stress markedly improved GR activity in rice seedlings. Above previous studies were focused on cereal crops, rice, and horticultural crops (Ma *et al.* 2016, Mahmud *et al.* 2017). However, there is still few reports about the effects of Cr stress on wheat crops. In our present study, the activity of AsA-GSH cycle in wheat seedlings leaves was enhanced to cope with the oxidative stress induced by Cr stress, which agreed with the results of Karuppanapandian *et al.*

Table 2. Effects of $NdCl_3$ on chlorophyll fluorescence parameters, net photosynthetic rate, and chlorophylls and carotenoids contents of wheat under Cr stress. The plants were treated as below. Control – half-strength Hoagland's solution; Nd – 30 μM $NdCl_3$; Cr – 80 mg L^{-1} $CrCl_3$; Nd + Cr – 30 μM $NdCl_3$ + 80 mg L^{-1} $CrCl_3$. The plants were firstly treated by $NdCl_3$ for 12 h, and then treated by Cr stress for 48 h. Car – carotenoids; F_v/F_m – maximum photochemical efficiency of PSII; P_N – net photosynthetic rate; q_N – nonphotochemical quenching; q_P – photochemical quenching; Φ_{PSII} – effective quantum yield of PSII. Values are means ± SD, $n = 6$. *Different lowercase letters* mean statistical difference at 5% level of significance.

Treatment	F_v/F_m	q_P	q_N	Φ_{PSII}	P_N [μmol m^{-2} s^{-1}]	Chl [mg g^{-1}(FM)]	Car [mg g^{-1}(FM)]
Control	0.80 ± 0.08^b	0.51 ± 0.07^b	0.21 ± 0.02^d	0.43 ± 0.06^b	7.00 ± 0.63^b	2.17 ± 0.27^b	0.63 ± 0.06^b
Nd	0.88 ± 0.10^a	0.58 ± 0.06^a	0.26 ± 0.03^c	0.50 ± 0.08^a	8.10 ± 0.90^a	2.43 ± 0.23^a	0.75 ± 0.09^a
Cr	0.57 ± 0.07^d	0.34 ± 0.04^d	0.33 ± 0.05^b	0.30 ± 0.04^d	4.70 ± 0.55^d	1.62 ± 0.15^d	0.42 ± 0.04^d
Nd + Cr	0.70 ± 0.09^c	0.42 ± 0.05^c	0.39 ± 0.04^a	0.36 ± 0.47^c	5.80 ± 0.64^c	1.86 ± 0.22^c	0.53 ± 0.06^c

Table 3. Effects of $NdCl_3$ on the contents of Nd and Cr in wheat roots and leaves under Cr stress. The plants were treated as in Table 2. The plants were firstly treated by $NdCl_3$ for 12 h, and then treated by Cr stress for 7 d. Values are means ± SD, $n = 6$. *Different lowercase letters* mean statistical difference at 5% level of significance.

Treatment	Nd content in leaves [mg kg^{-1}(DM)]	Nd content in roots [mg kg^{-1}(DM)]	Cr content in leaves [mg kg^{-1}(DM)]	Cr content in roots [mg kg^{-1}(DM)]
Control	0.16 ± 0.02^c	0.46 ± 0.05^c	3.00 ± 0.26^c	10.30 ± 1.30^c
Nd	4.40 ± 0.49^a	26.70 ± 3.15^a	2.50 ± 0.22^c	8.80 ± 0.74^c
Cr	0.13 ± 0.02^c	0.40 ± 0.04^c	41.00 ± 4.77^a	390.60 ± 47.80^a
Nd + Cr	3.20 ± 0.44^b	21.90 ± 1.83^b	27.20 ± 2.80^b	264.70 ± 33.16^b

Table 4. Effects of $NdCl_3$ on malondialdehyde (MDA) content, electrolyte leakage (EL), root length, plant height, and plant biomass of wheat under Cr stress. The plants were treated as in Table 2. The plants were firstly treated by $NdCl_3$ for 12 h, and then treated by Cr stress. After 48 h of treatment, MDA content was determined. After 7 d of treatment, plant height and biomass were determined. Values are means ± SD, $n = 6$. *Different lowercase letters* mean statistical difference at 5% level of significance.

Treatment	MDA content [nmol g^{-1}(FM)]	EL [%]	Plant height [cm]	Root length [cm]	Plant biomass [mg(FM) $plant^{-1}$]
Control	5.70 ± 0.66^c	12.80 ± 1.01^c	16.30 ± 1.37^b	13.70 ± 1.55^b	104.70 ± 9.46^b
Nd	4.30 ± 0.39^d	10.00 ± 0.86^d	18.80 ± 1.95^a	15.50 ± 1.80^a	118.50 ± 12.22^a
Cr	13.30 ± 1.17^a	29.50 ± 2.12^a	12.00 ± 1.41^d	9.50 ± 1.11^d	77.20 ± 6.19^d
Nd + Cr	9.00 ± 0.84^b	20.20 ± 1.83^b	14.20 ± 1.36^c	11.70 ± 1.33^c	90.80 ± 9.47^c

(2006), Farid *et al.* (2018), and Kabir (2016). Besides, our results showed that Cr stress markedly improved the activities of DHAR and MDHAR in wheat leaves. The present results indicated that wheat could fight against Cr stress through AsA-GSH cycle at physiological level. The redox state of plants has close relationships with AsA/DHA and GSH/GSSG, which can be modulated by AsA-GSH cycle. In this study, our present findings showed that Cr stress improved the activities of APX, GR, DHAR, and MDHAR. However, our study showed that Cr stress reduced AsA/DHA and GSH/GSSG, which was because of the oxidative stress induced by Cr stress. Liu *et al.* (2020) reported that Cr stress significantly downregulated the expression of genes involved in redox process and upregulated the expression of genes involved in the stress response in *Arabidopsis thaliana*, which was proved by our current results by analyzing the activities of enzymes responsible for the redox states of ascorbate and glutathione.

Previous studies showed that REEs La and Ce all increased AsA/DHA and GSH/GSSG ratios by activating AsA-GSH cycle in plants (Dai *et al.* 2017, Zheng and Guo 2018). Under heavy metal stress, Dai *et al.* (2017) showed that La improved cadmium tolerance of maize seedlings by regulating AsA/DHA and GSH/GSSG through the enhancement of AsA-GSH cycle. For current study, our results showed that Nd improved the activities of APX, GR, DHAR, and MDHAR, which further maintained AsA/DHA and GSH/GSSG and improved the antioxidant capacity of wheat plants. Thus, our results showed that Nd improved the antioxidant capacity of wheat crops by enhancing AsA-GSH cycle. These findings were the novelty of current study. Besides, previous studies showed that La and Ce had positive effects on stress tolerance of plants at low concentrations, but had negative effects at high concentrations. For the present study, we also found that Nd had the same effects as other REEs in previous studies. Therefore, the results of previous and our present

studies may confirm that low concentrations of REEs had positive effects on the stress tolerance of plants.

It has been documented that the function of chloroplasts was closely related with the antioxidant capacity and the contents of photosynthetic pigments (Chen and Shan 2019). For the present study, our results clearly showed that Nd partly alleviated the negative effects of Cr on the contents of photosynthetic pigments Chl and Car. This phenomenon indicated that Nd alleviated Cr-induced oxidative damage to photosynthetic pigments by enhancing the antioxidant capacity through AsA-GSH cycle. In addition, we found that Nd partly alleviated the negative effects of Cr stress on chloroplast function indicated by the Chl fluorescence parameters. In this study, Cr stress markedly reduced F_v/F_m, Φ_{PSII}, and q_P, which implied that Cr stress inhibited the electron transport and photochemical activity of PSII. While, Nd plus Cr stress markedly improved F_v/F_m, Φ_{PSII}, and q_P, which suggested that Nd promoted the photochemical conversion efficiency and photochemical activity of PSII in Cr-stressed wheat seedlings. However, Cr stress markedly improved q_N, which indicated that wheat crops enhanced the photoprotective capacity under Cr stress. Meanwhile, we found that Nd further enhanced the photoprotective capacity of wheat crops by improving q_N under Cr stress. Therefore, our findings indicated that Nd improved the function of chloroplast by enhancing AsA-GSH cycle, which further promoted the growth of wheat seedlings.

Many studies showed that the injury induced by Cr stress has a close relationship with its accumulation in the organs of plants (Habiba *et al.* 2019, Zhao *et al.* 2019). In this study, we also found that Cr stress stimulated its accumulation in the roots and leaves of wheat seedlings. Besides, we found that Nd markedly reduced Cr accumulation in the roots and leaves of wheat seedlings, which further reduced the inhibitory effects of Cr stress on wheat seedlings.

The present results suggested that $NdCl_3$ improved the antioxidant capacity and the function of photosystem by enhancing the activity of AsA-GSH cycle, which, in turn, protected wheat seedlings against Cr stress. Our results provided new information for the roles of Nd in regulating the antioxidant mechanism of wheat crops under Cr stress.

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