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EXOGENOUS APPLICATION OF SODIUM NITROPRUSSIDE ALLEVIATED CADMIUM INDUCED CHLOROSIS, PHOTOSYNTHESIS INHIBITION AND OXIDATIVE STRESS IN CUCUMBER

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Abstract

Cadmium (Cd) is one heavy metal which is toxic and can cause physiological disorder of plants. In the present experiment, effects of Sodium nitroprusside (SNP), a NO donor, on the phenotype, photosynthesis and oxidative stress of cucumber leaves were studied under Cd stress. Application of 100μM $CdCl_2$ induced obvious chlorosis of cucumber leaves with lower pigment content and lower net photosynthetic rate (P_N). Higher accumulations of H_2O_2 and thiobarbituric acid-reactive substances (TBARS) were observed in Cd-treated cucumber leaves, which indicated Cd induced oxidative stress to cucumber leaves. Application of 100μM SNP reversed the chlorosis and lower P_N induced by Cd treatment. Activities of reactive oxygen species (ROS) scavenging enzymes and antioxidant capacity expressed by α,α-diphenyl-β-picryllhydrazyl (DPPH) scavenging activity, chelating activity of ferrous ions and hydroxyl radical (OH·) scavenging activity were significantly increased by SNP under Cd stress. On the contrary, the above effects of SNP were not observed after the application of potassium ferrocyanide which is an analog of SNP that does not release NO. Therefore, it could be concluded that the NO from SNP might account for the alleviating effect of Cd stress on cucumber plants.

Introduction

Cadmium (Cd) is one of widespread heavy metals; it can be easily up-taken by plants and enter the food chain to be particularly dangerous for human health. The toxic levels of Cd may originate from soil with characteristics of abundant Cd or agricultural manufacturing, mining and other waste disposal practices, or from the application of Cd-containing pesticides and phosphate fertilizers (Radotic *et al*., 2000). Some investigations indicated that Cd led to deficiency of iron and induced the inhibition of chlorophyll biosynthesis which resulted in a decline of the photosynthetic rate (Wahid *et al*., 2008; Zulfqar *et al*., 2012). Like other stress, Cd toxicity also destroys the balance between reactive oxygen species (ROS) and its scavenger, and induces oxidative stress to plants (Markovska *et al*., 2009).

Nitric oxide (NO) is a small, ubiquitous, highly diffusible gaseous bioactive molecule. Its chemical properties make NO a versatile signal molecule that functions through interactions with cellular targets via either redox or additive chemistry (Lamattina *et al*., 2003). Recent studies provided increasing evidences that NO is involved in modulating plants tolerance to abiotic stress including salt stress, temperature stress, water stress and heavy metal toxicity (Mahmood *et al*., 2009; Siddiqui *et al*., 2011). Cucumber (*Cucumis sativus* L.) is one of the important vegetables in the world, and it is sensitive to Cd toxicity (Feng *et al*., 2010). It has been reported that NO was involved in cucumber tolerance to drought and salt stress (Arasimowicz-Jelonek & Foryszak-Wieczorek, 2009; Shi *et al*., 2007), however, to our knowledge, there are few investigations about NO regulating Cd toxicity in cucumber. In the present study, possible mechanism of exogenous SNP alleviating Cd toxicity in cucumber was studied by investigating photosynthetic parameters and antioxidant metabolism, especially activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) which are involved in the ROS scavenging.

Material and Methods

Cucumber (*Cucumis sativus* L. cv. Jinyou 4) seeds (Provided by Tianjin Cucumber Research Institute) were germinated on moisture filter paper in the dark at 28°C for 2 days, and germinated seedlings were transferred to the growth chamber filled with vermiculite and grown in greenhouse for 11 days. Then they were transplanted into 5L black plastic containers containing aerated full nutrient solution: 4 mM $Ca(NO_3)_2$, 4 mM KNO_3, 2.5 mM KH_2PO_4, 2 mM $MgSO_4$, 29.6 μM H_3BO_3, 10 μM $MnSO_4$, 50 μM Fe-EDTA, 1.0 μM $ZnSO_4$, 0.05 μM H_2MoO_4, 0.95 μM $CuSO_4$, with 3 seedlings per container. After 10 days of pre-culture, the treatments were started. The experimental design consisted of a control and three treatments (Cd: 100 μM $CdCl_2$ treatment; Cd+ SNP: 100 μM $CdCl_2$ +100 μM SNP treatment; Cd+SF, 100 μM $CdCl_2$ +100 μM potassium ferrocyanide treatment) and was arranged in a randomized, complete block design with three replicates. Single container was used as treatment unit. The plants were cultivated in a greenhouse where the day/night time and temperature were about 12h/12h and 23-28 /18-23 , following 12 days of treatment application, samples were collected for physiological parameters.

Determination of plant growth and Fe concentration: After 12 days of treatments, the cucumber plants were harvested, divided in to shoots and roots. The samples were dried at 70°C to constant weight and weighted. For determination of Fe concentrations, 0.3g mixed powders of dried leaves were transferred to Kjeldahl flask, in which 5mL 30% H_2O_2 and 10mL of concentrated H_2SO_4 solution were added, and the sample was digested until the solution become clear. Then it was transferred to the volumetric flask and volume up to 100 ml was made up with the twice-distilled water. The concentration of iron was determined by flame atomic absorption spectrometry (Konieczyński & Wesolowski, 2007).

Determination of H_2O_2 content: H_2O_2 content was determined according to the method reported by Patterson *et al*., (1984). The assay was based on the absorbance change of the titaniumperoxide complex at 415nm. Absorbance values were quantified using standard curve generated from known concentrations of H_2O_2.

Determination of lipid peroxidation: Lipid peroxidation (LPO) was estimated by measuring the concentration of thiobarbituric acid reactive substances (TBARS) using the thiobarbituric acid method described by Heath *et al*., (1968). 0.3g of leaves were ground with 3ml of 0.1% (w/v) trichloroacetic acid (TCA).The homogenate was centrifuged at 10,000× *g* for 10 min and 3 ml of 20% TCA containing 0.5% (w/v) 2-thiobarbituric acid (TBA) was added to 1ml of supernatant. The mixture was heated at 95°C for 30 min and the reaction was stopped by quickly placing in an ice-bath. The cooled mixture was centrifuged at 10,000 × *g* for 10 min, and the absorbance of the supernatant was recorded at 532 and 600 nm. After substrating the non-specific absorbance at 600 nm, the TBARS concentration was determined by its extinction coefficient of 155 mM^{-1} cm^{-1}.

Enzyme extraction: For enzyme assays, 0.3g leaves were ground with 3 ml ice-cold 25 mM HEPES buffer (pH 7.8) containing 0.2 mM EDTA, 2 mM ascorbate and 2% PVP. The homogenates were centrifuged at 4°C for 20 min at 12,000 × *g* and the resulting supernatants were used for determination of enzymatic activities (Zhu *et al*., 2004). All spectrophotometric analyses were conducted on a SHIMADZU UV-2450PC spectrophotometer.

Determination of antioxidant enzyme activities: SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium following the method of Stewart & Bewley (1980). CAT activity was determined as the decline in absorbance at 240 nm due to the decrease of extinction of H_2O_2 using the method of Patra *et al*., (1978). GPX activity was determined as the increase in absorbance at 470 nm due to guaiacol oxidation (Nickel *et al*., 1969). APX activity was determined by the decrease in absorbance at 290 nm as ascorbate was oxidized (Nakano *et al*., 1981). DHAR activity was assayed by measuring the increase in absorbance at 265nm due to reduced ascorbate formation (Nakano *et al*., 1981). GR activity was measured according to Foyer *et al*., (1976), which depended on the rate of decrease in the absorbance of NADPH at 340 nm.

Determination of photosynthetic parameters: The content of photosynthetic pigments was measured according to the method of Arnon (1949). Net photosynthetic rate was measured by a portable photosynthesis system Li-6400 (Li-Cor, Lincoln, USA) at ambient CO_2 concentration of 340μmol mol^{-1} and a photon flux density of 1000μmol m^{-2} S^{-1}. Fv/Fm was measured with a pulse amplitude modulated system (model FMS2, Hansatech Istrument, UK) according to Burzyński & Klobus (2004).

Determination of glutathione content: frozen sample (0.3g) was ground with a mortar and pestle in 3ml of 0.5mM EDTA solution containing 3% TCA at 4°C. The homogenate was centrifuged at 15,000×*g* for 10 min. Then 0.2mL supernatant was added to 1.5mL of 50mM potassium phosphate buffer (pH 7.0) containing 0.2mM 5,5/-dithio-bis (2-nitrobenzoic) (DTNB). The mixture was incubated at 30°C for 2 min. Absorbance was determined at 412nm and the glutathione (GSH) concentration was calculated by comparison to a standard curve (Ellman, 1959).

Determination of antioxidant activity: For determination of antioxidant activities indicated as DPPH scavenging capacity, Hydroxyl radical (OH) scavenging capacity and Fe^{2+}-chelating capacity, 0.3g sample was suspended in 3ml of serine borate buffer (100mM Tris-HCl, 10mM borate, 5mM serine, and 1 mM diethylenetriaminepentacetic acid, pH 7.0). The slurry was centrifuged at 5000 × *g* for 10 min at 4°C and the supernatants were used for the in vitro antioxidant assays. All samples were placed on ice during the experiments. The analysis of antioxidant capacity was done according to the method of Manda *et al*., (2010).

The statistics method: Values presented were means ± 1 standard deviation (SD) of three replicates. Statistical analyses were carried out by analysis of variance (ANOVA) using SPSS10.0 software. Differences between treatments were analyzed by the Ducan's multiple range test.

Results

Effects of exogenous SNP on leaf symptom, photosynthetic parameters and plant growth of cucumber under Cd stress: Cd stress dramatically induced cucumber leaf chlorosis, in accordance with the chlorosis, the contents of chlorophyll a, chlorophyll b and carotenoid were significantly decreased by Cd treatment ($p<0.05$) (Fig. 1 & Table 1). Application of SNP reversed the chlorosis of cucumber leaves induced by Cd stress, while application of potassium ferrocyanide, an analog of SNP that does not release NO, did not show significant effect on Cd-induced cucumber leaf chlorosis and lower pigment contents. Cd stress significantly inhibited the net photosynthetic rate (P_N) ($p<0.05$) and Fv/Fm ratio ($p<0.05$) and reduced stomatal conductance (G_s) ($p<0.05$), which were accompanied by higher intracellular CO_2 concentration (Ci) levels. SNP application significantly increased Gs and alleviated the inhibition of P_N and Fv/Fm by Cd treatment, and resulted in lower Ci. These effects of SNP on photosynthetic parameters were not obtained by application of potassium ferrocyanide. Consistent with the effects on pigments and photosynthesis, Cd stress significantly inhibited the growth of cucumber shoots and roots, and SNP application reduced the inhibition, while potassium ferrocyanide did not show obvious influence on cucumber plant growth under Cd stress (Fig. 3).

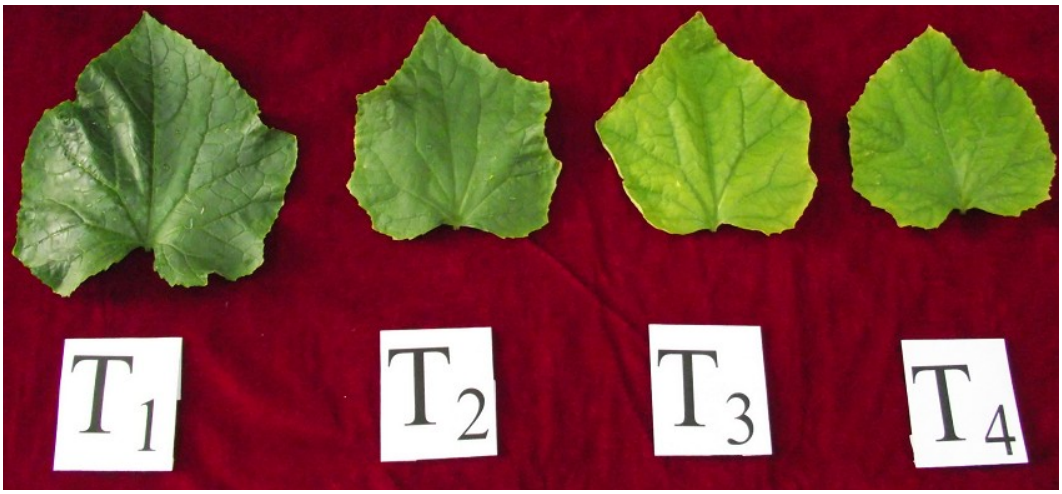

Fig. 1. Effects of exogenous sodium nitroprusside on the phenotype of Cd-treated cucumber leaves. T1, Control; T2, 100μM $CdCl_2$ + 100μM SNP; T3, 100μM $CdCl_2$; T4, 100μM $CdCl_2$ + 100 μM potassium ferrocyanide.

Table 1. Effects of exogenous sodium nitroprusside on pigment contents, P_N (net photosynthetic rate), E (transpiration rate), Ci (intracellular CO_2 concentration), g_s (stomatal conductance) and Fv/Fm in Cd-treated cucumber leaves.

Treatment	Parameters							
	Chl a	Chl b	Carotenoid	P_N	E	g_s	Ci	Fv/Fm
CK	1.41±0.05a	0.26±0.04a	0.36±0.02a	17.57±0.75a	10.15±0.67a	0.42±0.04a	295.0±7.00c	0.84±0.007a
Cd	0.68±0.07c	0.16±0.02c	0.18±0.01c	7.87±0.50c	2.76±0.44c	0.09±0.01c	353.7±4.93a	0.75±0.011c
SNP	0.94±0.07b	0.19±0.02b	0.29±0.04b	12.13±0.70b	6.48±0.79b	0.29±0.05b	318.7±2.89b	0.80±0.011b
SF	0.70±0.06c	0.15±0.03c	0.19±0.02c	8.2±1.06c	2.83±0.62c	0.10±0.02c	345.3±11.93a	0.76±0.013c

Note: Data are means ± SD of three replicates. Mean values followed by different letters (a-c) are significantly different (p<0.05). CK, Control; Cd, 100 μM $CdCl_2$; SNP, 100 μM $CdCl_2$ + μM SNP; SF, 100 μM $CdCl_2$ + 100 μM potassium ferrocyanide. Units of Chl a, Chl b and carotenoid were mg g^{-1} FW. Units of P_N, E, g_s and Ci were μmol m^{-2} s^{-1}, mmol m^{-2} s^{-1}, mol m^{-2} s^{-1} and μL L^{-1}, respectively

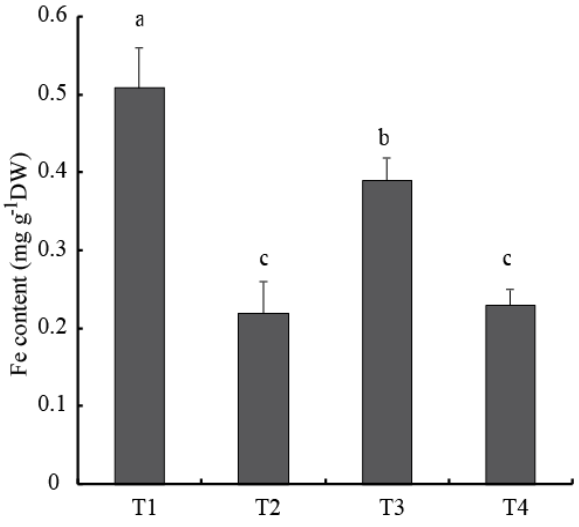

Fig. 2. Effects of exogenous sodium nitroprusside on the Fe content in Cd-treated cucumber leaves. Data are means ± SD of three replicates. Mean values followed by different letters (a-c) are significantly different (p<0.05). T1, Control; T2, 100μM $CdCl_2$; T3, 100μM $CdCl_2$ +100μM SNP; T4, 100μM $CdCl_2$ + 100μM potassium ferrocyanide.

Effects of exogenous SNP on Fe content in cucumber leaves under Cd stress: Compared to control, Cd treatment significantly reduced Fe accumulation in cucumber leaves (p<0.05), and application of SNP significantly increased Fe content of cucumber leaves (p<0.05), while no similar results were observed by application of potassium ferrocyanide under Cd stress (Fig. 2).

Effects of exogenous SNP on H_2O_2 and lipid peroxidation of cucumber leaves under Cd stress: Compared to the control, Cd treatment dramatically induced accumulation of H_2O_2 (p<0.05), in accordance with higher H_2O_2 concentration, TBARS, as an indicator of lipid peroxidation level, were significantly increased in cucumber leaves under Cd stress (p<0.05). The excessive H_2O_2 accumulation of cucumber leaves induced by Cd were dramatically scavenged by application of SNP (p<0.05), which also resulted in lower lipid peroxidation. The effects of SNP on H_2O_2 and lipid peroxidation could not be gotten by application of potassium ferrocyanide (Fig.4).

Effects of exogenous SNP on antioxidant system in cucumber leaves under Cd stress: As shown in Table 2, Cd stress significantly inhibited the activities of

SOD, CAT, GPX, APX, DR and GR in cucumber leaves ($p<0.05$). Application of exogenous SNP increased activities of all antioxidant enzymes under Cd stress, while application of potassium ferrocyanide did not significantly affected activities of these antioxidant enzymes.

DPPH scavenging capacity, ·OH scavenging capacity and metal chelating capacity were usually used to express antioxidant capacity of plant tissues. Compared to the control, Cd stress dramatically decreased antioxidant capacity of cucumber leaves, and reduced DPPH scavenging capacity, ·OH scavenging capacity and metal chelating capacity by 68%, 35%, 43%, respectively. While application of exogenous SNP significantly alleviated the inhibition of antioxidant capacity by Cd stress, and increased DPPH scavenging capacity, ·OH scavenging capacity and metal chelating capacity by 100, 33 and 61% compared to the Cd stress, respectively. The effects of exogenous SNP increasing antioxidant capacity under Cd stress were not found by application of potassium ferrocyanide (Table 2).

Glutathione (GSH), as an important antioxidant, was significantly reduced in cucumber leaves under Cd stress, in which GSH content was only 56% of control ($p<0.05$). Application of SNP led to significant elevation of GSH content under Cd stress, and 63% higher than Cd treatment was observed ($p<0.05$). Like other parameters, application of potassium ferrocyanide did not significantly GSH content in cucumber leaves under Cd stress, too (Table 2).

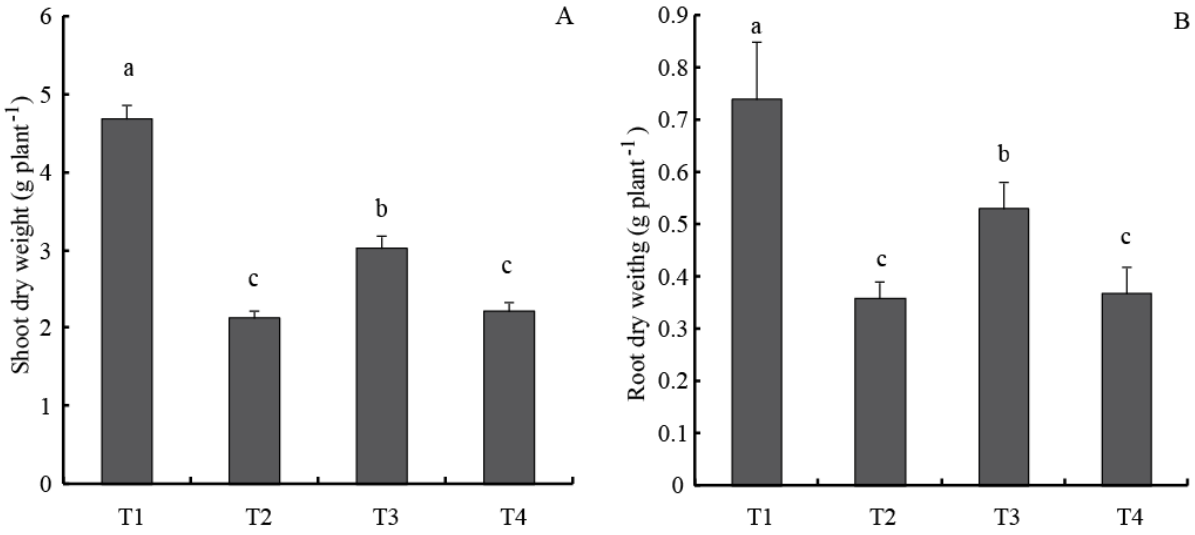

Fig. 3. Effects of exogenous sodium nitroprusside on the dry weigh of shoot (A) and root (B) in Cd-treated cucumber plants. Data are means ± SD of three replicates. T1, Control; T2, 100μM $CdCl_2$; T3, 100μM $CdCl_2$ +100μM SNP; T4, 100μM $CdCl_2$ + 100μM potassium ferrocyanide.

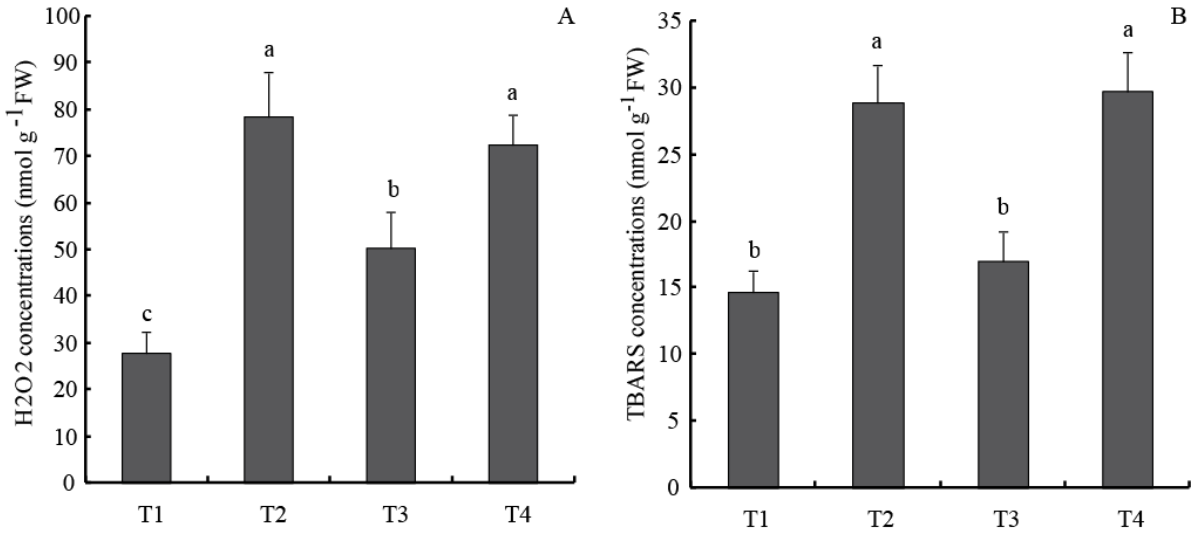

Fig. 4. Effects of exogenous sodium nitroprusside on concentrations of H_2O_2 (A) and TBARS (B) in Cd-treated cucumber leaves. Data are means ± SD of three replicates. Mean values followed by different letters (a-c) are significantly different ($p<0.05$). T1, Control; T2, 100μM $CdCl_2$; T3, 100μM $CdCl_2$ + 100μM SNP; T4, 100μM $CdCl_2$ + 100μM potassium ferrocyanide.

Table 2. Effects of exogenous sodium nitroprusside on activities of SOD, CAT, GPX, APX, DR and GR, DPPH scavenging capacity, ·OH scavenging capacity, metal chelating capacity and GSH contents in Cd-treated cucumber leaves.

Treatment	Parameters									
	SOD activity	CAT activity	GPX activity	APX activity	DR activity	GR activity	DPPH scavenging capacity	·OH scavenging capacity	Metal chelating capacity	GSH content
CK	38.29±3.06a	5.69±0.53a	21.00±3.14a	7.32±0.61a	0.88+0.12a	0.54±0.04a	29.26±3.4a	45.64±2.98a	36.10±2.49a	10.53±1.15a
Cd	15.60±3.18c	2.27±0.14c	8.83±1.59c	3.16±0.29c	0.18±0.02c	0.09±0.02c	9.33±1.45c	29.56±2.49c	20.71±1.90b	5.86±0.82b
SNP	27.29±2.17b	3.61±0.42b	15.84±1.30b	4.83±0.29b	0.35±0.03b	0.26±0.03b	18.65±1.84b	39.02±3.93b	33.41±2.66a	9.57±0.54a
SF	16.62±1.46c	2.37±0.27c	10.01±0.67c	3.32±0.31c	0.20±0.04c	0.11±0.01c	10.65±1.76c	28.70±3.37c	17.47±2.13b	5.17±0.77b

Note: Data are means ± SD of three replicates. Mean values followed by different letters (a-c) are significantly different ($p<0.05$). CK, Control; Cd, 100 μM $CdCl_2$; SNP, 100 μM $CdCl_2$ + 100 μM SNP; SF, 100 μM $CdCl_2$ + 100 μM potassium ferrocyanide. Units of SOD, CAT, GPX, APX, DR and GR activities were U mg^{-1} protein, μmol H_2O_2 mg^{-1} protein min^{-1}, μmol guaiacol mg^{-1} protein min^{-1}, μmol AsA mg^{-1} protein min^{-1}, μmol DHA mg^{-1} protein min^{-1} and μmol NADPH mg^{-1} protein min^{-1}, respectively. DPPH scavenging capacity, ·OH scavenging capacity and metal chelating capacity were expressed by percentage (%). The unit of GSH content was nmol g^{-1}FW

Discussion

Cadmium is one of the most common heavy metal pollutant in worldwide soil, and greatly limits crop productivity because of its easy accumulation within plant tissues and its interference with essential physiological processes, among which Fe deficiency induced by Cd was considered to be mainly responsible for chlorosis (Gao *et al*., 2011). In the present study, obvious chlorosis and lower Fe content were also observed in Cd-treated cucumber leaves. In accordance with the chlorosis, the pigments were significantly reduced and resulted in lower P_N which led to the inhibition of cucumber plant growth. In *Brassica napus*, *Brassica juncea* and *Lepidium sativum* L. pigment contents, photosynthesis and plant growth were also significantly reduced by Cd treatments (Raziuddin *et al*., 2011; Gill *et al*., 2012), and Gill *et al*., (2012) thought that the physiological disorder greatly depended on nitrogen metabolism perturbation by Cd. Accompanied with the reduction of P_N, lower Gs was simultaneously observed in cucumber leaves, nevertheless, the reduction of P_N did not result from a low stomatal conductance (G_s) and low CO_2 concentration in chloroplasts, because the intracellular CO_2 concentration (Ci) levels were much higher in leaves treated with Cd compared to the control (Table 1). Based on this, it could be concluded the inhibition of photosynthetic processes by Cd in cucumber was attributed to nonstomatal restriction (Feng *et al*., 2010). The Fv/Fm ratio is used to express the photochemical efficiency of PSII, in the present study, Cd treatment caused significant decrease of Fv/Fm, which is a reliable sign of photoinhibition (Krause, 1988), and at the same time, lower Fv/Fm also showed that the ability of PSII reducing the primary acceptor Q_A was inhibited by Cd (Babani *et al*., 1996).

Nitric oxide, as a gas signal molecule, can readily form complexes with transition metal ions in aqueous solutions or those present in diverse nucleophylic compounds such as metalloproteins (Stamler *et al*., 1992). The Fe (III)NO complex appears to undergo a charge transfer reaction to form $Fe(II)NO^+$ and NO could increase the availability of iron in plants (Graziano *et al*., 2002). In the present experiment, application of SNP reversed the chlorosis of cucumber leaves induced by Cd stress. As NO donor SNP contains iron in its molecule, it is important to exclude the effect of iron in SNP for the elucidation of NO function. Therefore, potassium ferrocyanide, an analog of SNP that does not release NO was used to treat cucumber instead The results showed that it had no significant effect on Fe content and Cd-induced cucumber leaf chlorosis. Therefore, NO from SNP increasing the Fe content might play important role in alleviation of cucumber leaf chlorosis induced by Cd. There were some other investigations indicated that NO was a key component in the regulation of iron uptake and homeostasis in plants (Chen *et al*., 2010b; Graziano & Lamattina, 2007), In accordance with the chlorosis alleviation by application of SNP, photosynthesis of cucumber was significantly prompted and plant growth was increased, similar function of SNP has been observed in Cd-treated barley (Chen *et al*., 2010a).

Excessive production of reactive oxygen species (ROS) was found in several plant treated with Cd (Markovska *et al*., 2009; Chen *et al*., 2010a; Dai *et al*., 2012). In the experiment, Cd treatment dramatically induced accumulation of H_2O_2. A direct result of excessive ROS is lipid peroxidation which is often indicated as TBARS contents, and the accumulation of TBARS was considered to be caused by oxidative degradation of polyunsaturated fatty acids, in particular linolenic acid which is localized mainly in the thylakoid glycolipids, therefore, excessive TBARS accumulation in Cd treated cucumber leaves is likely a good measure for peroxidative damage to chloroplast membrane (Van-Hasselt *et al*., 1996).

It is well known higher efficacy of antioxidant system plays an important role in keeping ROS balance in plant tissues under stress conditions(Kafi *et al*., 2011). In the present study, we found that Cd dramatically inhibited activities of antioxidant enzymes and lowered GSH contents, which could mainly be responsible for oxidative stress induced by Cd. Above results indicated that exogenous SNP could reduce Cd-induced oxidative stress. To confirm the possible mechanism, antioxidant capacities were determined in the present study. It was observed that exogenous SNP significantly reversed the inhibition of all antioxidant capacities indicated as DPPH-radical scavenging activity, hydroxyl radical scavenging activity and the chelating activity in cucumber leaves under Cd stress. Increase of antioxidant capacity in plants usually depends on antioxidant metabolites such as glutathione, polyphenols and flavonoids (Ksouri *et al*., 2007), and

there were reports indicating that exogenous NO could induce synthesis of these antioxidant metabolites (Ksouri *et al*., 2007; Ferreira *et al*., 2010; Wu *et al*., 2007), especially glutathione, it could be increased by NO modulating both synthesis pathway (Innocenti *et al*., 2007; Moellering *et al*., 1998) and reduction pathway (Xu *et al*., 2010). Glutathione (GSH) content in cucumber leaves was increased by exogenous SNP treatment under Cd stress condition, this should be one important reason that exogenous SNP increased antioxidant capacity of cucumber leaves under Cd stress. Except for antioxidant substances, several ROS-scavenging enzymes including SOD, CAT, GPX, APX, DR and GR play important role in alleviating oxidative stress (Nabati *et al*., 2011). Cheng *et al*., (2002) reported that the inhibition of polyethylene glycol (PEG)- and dehydration (DH)-enhanced senescence of rice leaves by NO was most likely modulated through increasing SOD activity which resulted in lower lipid peroxidation. In the present experiment, exogenous SNP significantly alleviated the inhibited level of SOD activity by Cd stress (Table 2), which suggested that application of SNP could promote the conversion from $O_2^{\cdot-}$ to H_2O_2 and O_2 in cucumber leaves under Cd stress. It is well known that SOD includes Mn-SOD, Cu,Zn-SOD and Fe-SOD, and it could be able to conclude that the increasing SOD activity by exogenous SNP might be partly attributed to higher Fe efficiency. H_2O_2, as the products of $O_2^{\cdot-}$ decomposition, can rapidly diffuse across the membrane and is toxic because it acts both as an oxidant as well as reductant (Foyer, 1994), under such situation, the highly efficiency of H_2O_2 scavenging is very vital. In our experiment, exogenous SNP significantly increased CAT and GPX activities (Table 2), which can directly scavenge H_2O_2 and play important roles in reducing oxidative stress induced by abiotic stress, similar results were obtained in heat stressed chrysanthemum (Yang *et al*., 2011). Besides SOD, CAT and GPX, there exists another important ROS-scavenging system which is ascorbate-glutathione cycle in chloroplast (Murshed *et al*., 2008). The cycle is mainly composed of enzymes such as APX, DR, GR and antioxidants such as glutathione and ascorbate. In our experiment, exogenous SNP significantly induced activities of APX, DHAR and GR (Table 2), which are very important in protecting chloroplast from oxidative damage and might be greatly responsible for the higher photosynthesis capacity of Cd-treated cucumber in the present study.

Conclusion

Exogenous SNP application effectively revered the chlorosis and alleviating growth inhibition of cucumber induced by Cd stress, while application of potassium ferrocyanide, an analog of SNP that does not release NO, did not showed the effects. Based on the results, it could conclude that the effects of SNP alleviating Cd stress on cucumber was due to NO from it, and the mechanism might depend on higher Fe efficiency and antioxidant efficiency induced by SNP.

Acknowledgements

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References

Arasimowicz-Jelonek, M., J.F. Wieczorek and J. Kubiś. 2009. Involvement of nitric oxide in water stress-induced responses of cucumber roots. *Plant Sci*., 177: 682-690.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1-15.

Babani, F. and H.K. Lichtenthaler. 1996. Light-induced and age-dependent development of chloroplasts in etiolated barley leaves as visualized by determination of photosynthetic pigments, CO_2 assimilation rates and different kinds of chlorophyll fluorescence ratios. *J. Plant Physiol*., 148: 555-566.

Burzyński, K. and G. Klobus. 2004. Changes of photosynthetic parameters in cucumber leaves under Cu, Cd, and Pb stress. *Photosynthetica*, 42: 505-510.

Chen, F., F. Wang, H. Sun, Y. Cai, W. Mao, G. Zhang, E. Vincze and F. Wu. 2010a. Genotype-dependent effect of exogenous nitric oxide on Cd-induced changes in antioxidative metabolism, ultrastructure, and photosynthetic performance in barley seedlings (*Hordeum vugare*). *J. Plant Growth Regul.,* 29: 394-408.

Chen, W.W., J.L. Yang, C. Qin, C.W. Jin, J.H. Mo, T. Ye and S.J. Zheng. 2010b. Nitric oxide acts downstream of auxin to trigger root ferric-chelate reductase activity in response to iron deficiency in *Arabidopsis*. *Plant Physiol*., 154: 810-819.

Cheng, F.Y., S.Y. Hsu and C.H. Kao. 2002. Nitric oxide counteracts the senescence of detached rice leaves induced by dehydration and polyethylene glycol but not by sorbitol. *Plant Growth Regul.*, 38: 265-272.

Dai, H., C. Shan, C. Lu, G. Jia, A.Wei, S. Wenqing and T. Yang. 2012. Response of antioxidant enzymes *Populus×Canescens* under cadmium stress. *Pak. J. Bot*., 44(6): 1943-1949.

Ellman, G.L. 1959. Tissue sulfydryl groups. *Arch. Biochem. Biophys.*, 82: 70-77.

Feng, J., Q. Shi, X. Wang, M. Wei, F. Yang and H. Xu. 2010. Silicon supplementation ameliorated the inhibition of photosynthesis and nitrate metabolism by cadmium (Cd) toxicity in *Cucumis sativus* L. *Sci. Hort.*, 123: 521-530.

Ferreira, L.C., A.C. Cataneo, L.M.R. Remaeh, N. Corniani, T. de Fátima Fumis, Y.A. de Souza, J. Scavroni and B.J.A. Soares. 2010. Nitric oxide reduces oxidative stress generated by lactofen in soybean plants. *Pestic. Biochem. Physiol*., 97: 47-54.

Foyer, C.H. and B. Halliwell. 1976. The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. *Planta*, 133: 21-25.

Foyer, C.H.1994. Photooxidative stress in plants. *Physiol. Plant*., 92: 696-717.

Gao, C., Y. Wang, D.S. Xiao, C.P. Qiu, D.G. Han, X.Z. Zhang, T. Wu and Z.H. Han. 2011. Comparison of cadmium-induced iron-deficiency responses and genuine iron-deficiency responses in *Malus xiaojinensis*. *Plant Sci*., 181: 269-274.

Gill, S.S., N.A. Khan and N. Tuteja. 2012. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). *Plant Sci*., 182: 112-120.

Graziano, M. and L. Lamattina. 2007. Nitric oxide accumulation is required for molecular and physiological responses to iron deficiency in tomato roots. *Plant J*., 52: 949-960.

Graziano, M., M.V. Beligni and L. Lamattina. 2002. Nitric oxide improves internal iron availability in plants. *Plant Physiol*., 130: 1852-1859.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.*, 125: 189-198.

Innocenti, G., C. Pucciariello, M.L. Gleuher, J. Hopkins, M. de Stefano, M. Delledonne, A. Puppo, E. Baudouin and P. Frendo. 2007. Glutathione synthesis is regulated by nitric oxide in *Medicago truncatula* roots. *Planta*, 225: 1597-1602.

Kafi, M., J. Nabati, A. Masoumi and M.Z. Mehrgerdi. 2011. Effect of salinity and silicon application on oxidative damage of sorghum [*Sorghum Bicolor* (L.) Moench.]. *Pak. J. Bot*., 43(5): 2457-2462.

Konieczyński, P. and M. Wesolowski. 2007. Determination of zinc, iron, nitrogen and phosphorus in several botanical species of medicinal plants. *Polish J. Environ. Stud*., 16: 785-790.

Krause, G.K.Z. 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protetive mechanisms. *Physiol Plant*., 74: 566-574.

Ksouri, R., W. Megdiche, A. Debez, H. Falleh, C. Grignon and C. Abdelly. 2007. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritime*. *Plant Physiol. Biochem*., 45: 244-249.

Lamattina, L., C. García-Mata, M. Graziano and G. Pagnussat. 2003. Nitric oxide: the versatility of an extensive signal molecule. *Annu. Rev. Plant Biol*., 54:109-136.

Mahmood, T., K.J. Gupta and W.M. Kaiser. 2009. Cadmium stress stimulates nitric oxide production by wheat roots. *Pak. J. Bot.*, 41: 1285-1290.

Manda, K.D., C. Adams and N. Ercal. 2010. Biologically important thiols in aqueous extracts of spices and evaluation of their in vitro antioxidant properties. *Food Chem*., 118: 589-593.

Markovska, Y.K., N.I. Gorinova, M.P. Nedkovska and K.M. Miteva. 2009. Cadmium-induced oxidative damage and antioxidant responses in *Brassica juncea* plants. *Biol Plant*., 53: 151-154.

Moellering, D., J. McAndrew, R.P. Patel, T. Cornwell, T. Lincoln, X. Caom, J.L. Messina, H.J. Forman, H. Jo and V.M. Darley-Usmar. 1998. Nitric oxide-dependent induction of glutathione synthesis through increased expression of γ-glutamylcysteine synthetase. *Arch. Biochem. Biophy*., 358: 74-82.

Murshed, R., F. Lopez-Lauri and H. Sallanon. 2008. Microplate quantification of enzymes of the plant ascorbate-glutathione cycle. *Anal. Biochem*., 383: 320-322.

Nabati, J., M. Kafi, A. Nezami, P.R. Moghaddam, A. Masoumi and M.Z. Mehrjerdi. 2011. Effect of salinity on biomass production and activties of some key enzymatic antioxidants in kochia (*Kochia Scoparia*). *Pak. J. Bot*., 43(1): 539-548.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant Cell Physiol*., 22: 867-880.

Nickel, R.S. and B.A. Cunningham. 1969. Improved peroxidase assay method using Ieuco 2,3,6-trichlcroindophenol and application to comparative measurements of peroxidase catalysis. *Ann. Biochem.*, 27: 292-299.

Patra, H.L., H. Kar and D. Mishre. 1978. Catalase activity in leaves and cotyledons during plant development and senescence. *Biochem. Physiol*., 172: 385-390.

Patterson, B.D., E.A. Macrae and I.B. Ferguson. 1984. Estimation of hydrogen peroxide in plants extracts using titanium (IV). *Ann. Biochem*., 134: 487-492.

Radotic, K., T. Ducic and D. Mutavdzic. 2000. Changes in peroxidase activity and isoenzymes in spruce needles after exposure to different concentrations of cadmium. *Environ. Exp. Bot*., 44: 105-113.

Raziuddin, Farhatullah, G. Hassan, M. Akmal, S.S. Shah, F. Mohammad, M. Shafi, J. Bakht and W. Zhou. 2011. Effects of cadmium and salinity on growth and photosynthesis parameters of *Brassica* species. *Pak. J. Bot.*, 43(1): 333-340.

Shi, Q., F. Ding, X. Wang and M. Wei. 2007. Exogenous nitric oxide protect cucumber roots against oxidative stress induced by salt stress. *Plant Physiol. Biochem*., 45: 542-550.

Siddiqui, M.H., M.H. Al-Whaibi and M.O. Basalah. 2011. Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma*, 248: 447-455.

Stamler, J.S., D.J. Singel and J. Loscalzo. 1992. Biochemistry of nitric oxide and its redox-activated forms. *Science*, 258: 1898-1902.

Stewart, R.C. and J.D. Bewley. 1980. Lipid peroxidation associated with accelerated aging of soybean axes. *Plant Physiol*., 65: 245-248.

Van-Hasselt, P.R., W.S. Chow and J.M. Anderson. 1996. Short-term treatment of pea leaves with supplementary UV-B at different oxygen concentrations: impacts on chloroplast and plasma membrane bound processes. *Plant Sci*., 120: 1-9.

Wahid, A., A. Ghani and F. Javed. 2008. Effect of cadmium on photosynthesis, nutrition and growth of mungbean. *Agron. Sustain. Dev*., 28: 273-280.

Wu, C.H., R.K. Tewari, E.J. Hahn and K.Y. Paek. 2007. Nitric oxide elicitation induces the accumulation of secondary metabolites and antioxidant defense in adventitious roots of *Echinacea purpruea*. *J. Plant Biol*., 50: 636-643.

Xu, Y., X. Sun, J. Jin and H. Zhou. 2010. Protective effect of nitric oxide on light-induced oxidative damage in leaves of tall fescue. *J. Plant Physiol*., 167: 512-518.

Yang, W., Y. Sun, S. Chen, J. Jiang, F. Chen, W. Fang and Z. Liu. 2011. The effects of exogenously applied nitric oxide on photosynthesis and antioxidant activity in heat stressed chrysanthemum. *Biol. Plant*., 55: 737-740.

Zhu, Z., G. Wei, J. Li, Q. Qian and J. Yu. 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt stressed cucumber (*Cucumis sativus* L.). *Plant Sci.*, 167: 527-533.

Zulfqar, S., A. Wahid, M. Farooq, N. Maqbool and M. Arfan. 2012. Phytoremediation of soil cadmium using *Chenopodium* species. *Pak. J. Agri. Sci.*, 49: 435-445.

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