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NITRIC OXIDE MEDIATES THE STRESS RESPONSE INDUCED BY CADMIUM IN MAIZE PLANTS

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Summary: The putative physiological roles of nitrogen oxide (NO) in young maize plants and its potential ability to enhance plant tolerance to environmental constrains, like heavy metal toxicity were studied. Cadmium (Cd) stress decreased the shoot and root length and chlorophyll content, reduced the photosynthetic rate, transpiration and stomatal conductance. The exogenous simultaneous application of sodium nitroprusside, the NO donor, to Cd-treated plants recovered to high extent the rate of photosynthesis, transpiration and chlorophyll content. NO mitigated the cadmium damage to photosystem II reaction center by increasing the maximum quantum yield and markedly enhancing the effective quantum yield. The membrane permeability was restored. The data suggest that nitric oxide may protect cells by exerting ameliorating effect on Cd toxicity and partially restore photosynthesis.

Keywords: Cadmium; nitric oxide; photosynthesis; Zea mays L.

Abbreviations: PS II – photosystem 2; F_v/F_m – maximum quantum yield of PS II; NPQ – nonphotochemical quenching; PEPC – phosphoenol pyruvate carboxylase; RuBPC – ribulose 1,5-bisphosphate carboxylase; RWC – relative water content; SNP – sodium nitroprusside; WUE – water use efficiency.

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INTRODUCTION

Increase pollution soil due continuous use of heavy metal to contaminated industrial effluents is critical for crop production globally. The heavy metal greatly accumulates in soil and causes toxicity to plants. Cadmium is easily taken up by plant roots and can be loaded into the xylem for its transport into leaves and then it is transferred to animals and humans through the food chain. Cd induces genetic and biochemical changes in plant metabolism that are related to general and Cd-specific stress responses. Cadmium disturbs mineral nutrition (Sandalio et al 2001), water balance (Barceló and Poschenrieder 1990), root morphology, root and leaf anatomy, functionality and lipid composition of membranes (Quariti et al 1997, Hernandez and Cooke 1997, Popova et al 2009,

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Tran and Popova 2013). In addition, Cd causes a decrease in stomatal density and conductance to CO₂ (Khudsar et al 2001) and reduces the number of open stomata (Greger and Johansson 1992), which would further affect the rate of photosynthesis. In contrast, Baryla et al (2001) and Tran et al (2013) reported increased stomatal density in leaves exposed to Cd. As regard the primary target of cadmium inhibition, it is still not identified. It was demonstrated that the oxygen evolving complex (OEC) of photosystem 2 (PS II) is affected by Cd by replacing the Mn²⁺ ions at OEC, thereby inhibiting the reaction of PS II (Atal et al 1991, Yordanova et al 2009). Treatment with Cd²⁺ ions also impairs the electron transport system on the reducing side of PS II (Atal et al 1991, Sigfridson et al 2004). Negative effects of Cd can also be observed in the carboxylating phase of photosynthesis. The main target of the influence of Cd are two key enzymes of CO₂ fixation, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPC) (Krantev et al 2008, Popova et al 2009).

Cd toxicity is often associated with the oxidative stress, caused by an excessive formation of free oxygen radical production (Sanita di Toppi and Gabrielli 1999) and by modified activity of various antioxidant enzymes (Hegedüs et al 2001, Krantev et al 2008, Popova et al 2009). A first barrier against Cd stress is retention of Cd in the cell wall (Nishizono et al 1989).

Survival under stressful conditions depends on the plants ability to perceive the stimulus, generate and transmit signals, and induce biochemical changes that adjust the metabolism accordingly. Therefore, the search for signal molecules that mediate the stress tolerance is an important step in our better understanding how plants acclimate to the adverse environment. Accumulating evidence shows that nitric oxide (NO) is an important signal molecule involved in plant responses to biotic and abiotic stresses (Delledonne et al 1998). NO is classified as a phytohormone that might function as a gaseous endogenous plant growth regulator, as well as a nontraditional plant growth regulator (reviewed in Beligni and Lamatina 2001, Popova and Tuan 2010).

In recent years, significant progress has been made in elucidating the diverse biological activities of NO in plants. Treatment with sodium nitroprusside (SNP) as a donor of NO increases the rate of photosynthesis, chlorophyll content, transpiration rate and stomatal conductance in cucumber seedlings (Fan et al 2007). Nitric oxide has been found to enhance chlorophyll content in potato, lettuce, Arabidopsis, and in maize leaves (Graziano et al 2002). Furthermore, NO was suggested to be involved in the responses to drought (Neill et al 2002), salinity (Zhao et al 2004), cadmium (Hsu and Kao 2004) and herbicide stress (Beligni and Lamattina 1999). Tran et al (2013) demonstrated that NO had a beneficial effect on leaf structure and guard cell morphology, partially alleviating the negative effect of Cd on pea plants. Treatment with the NO donor SNP together with Cd, mitigated Cd-induced abnormalities in leaf development and structure, as well as protected guard cell morphology, which could lead to improved leaf functioning. The results indicated that exogenous NO can effectively facilitate structural adjustments in pea leaves under Cd stress, thus improving stress tolerance at the whole plant level.

This study was undertaken to investigate possible mediatory role of nitric oxide in photosynthesis under cadmium toxicity in young maize plants. Changes in some leaf gas exchange parameters, the activity of carboxylating enzymes RuBPCase and PEPCase, and some parameters related to the activity of PS II were assessed since they are known to be mostly affected by cadmium treatment.

MATERIALS AND METHODS

Plant growth and treatment with Cd

Seeds of maize (Zea mays L., var. Kneja 611) were surface sterilized, soaked in tap water for 6 h, and allowed to germinate on moist filter paper in the dark for 3 days. Then, seedlings were placed in polyethylene boxes filled with 1.8 L Hoagland solution. CdCl, was added to growth solution at concentrations of 25, 50, 75 and 100 µM. Treatments with 50 µM and 75 µM CdCl, were selected, which demonstrated the toxic nature of Cd, but allowed the growth of the seedlings to a third leaf stage. The concentration of SNP was 100 μ M. Besides the individual treatments mentioned above, simultaneous addition of 50 or 75 µM Cd and 100 µM SNP to the nutrient solution was conducted. The corresponding controls were grown in Hoagland solution. The plants were grown in a growth chamber at 22/18°C day/night temperatures and a 16/8 hours light/dark photoperiod, relative humidity between 50% and 60%, and 180 μ mol m⁻² s⁻¹ photosynthetic active radiations (PAR). The nutrient solution was continuously aerated and changed every 3 days. Plants were harvested 14 days after planting and used for analyses of leaves and roots.

Gas exchange

Gas exchange measurements were performedby using portable photosynthesis system Lcpro + (LCD BioScientific Ltd, Herfordshire, UK). Leaves of 5 - 6 plants were placed in the chamber. Quantum flux density was 550 μ mol m⁻²s⁻¹PAR, ambient CO₂ concentration was 400 ppm (±10), leaf temperature was 25 ± 2°C.

Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence from the upper leaf surface was measured with a pulse amplitude modulation fluorometer (Imaging - PAM M-Series, Walz, Effeltrich, Germany). F_v/F_m is the maximum quantum yield of PS II photochemistry and was calculated as F./ $F_m = (F_m - F_0)/F_m$, where F_m is the maximal fluorescence induced by a saturating pulse (8000 μ mol m⁻²s⁻¹ PPFD for 0.8s) in the dark-adapted leaves for 20 min, F₀ is the minimal fluorescence yield in the dark. The effective quantum yield of PS II, $\Delta F/$ F_{m} means the proportion of absorbed light energy used in photochemistry. $\Delta F/Fm' =$ $(F_{m}' - F)/F_{m}'$, where F is fluorescence of light adapted samples, F_m' is the maximal light adapted fluorescence yield when a saturating light pulse is superimposed on the steady state level. Excess energy absorbed by plants and dissipated by nonphotochemical quenching NPQ was calculated from the equation: $NPQ = (F_m)$ $-F_{m}')/F_{m}'$.

Enzyme extraction and assays

RuBPC (E.C. 4.1.1. 39) and PEPC (EC 4.1.1.31) activities were assayed from the activated crude preparation by following the incorporation of NaH¹⁴CO₃ into acid stable products (Popova et al 1988).

Electrolyte leakage assay

The degree of membrane integrity was assessed by the leakage of electrolytes from the whole above ground part of three plants with approximately similar sizes. The leaf segments were strictly washed, blotted dry, weighted and put in stopped vials filled with the exact volume of bidistilled and deionized water. The vials were than incubated for 20 hours in the dark with continuously shaking. The amount of electrolyte leakage was measured conductometrically.

Relative water content

Relative water content (RWC) was measured as described by Morgan (1986). The RWC values were calculated by the formula: RWC(%) = $(FW - DW)/(TW - DW) \times 100$, where FW is the leaf fresh weight, TW is leaf weight at fully hydrated state and DW is leaf dry weight.

Estimation of total chlorophyll

Chlorophyll content was estimated by CCM-300 portable chlorophyll meter (Opti-Sciences, Inc. USA). Five leaves from each treatment were measured and data was presented as mg Chlorophyll.dm⁻². Major veins and areas of obvious visual damage were avoided.

Determination of Cd content

Around 1 g of dry root and leaf material was wet digested in H_2SO_4 : HNO₃ mixture (1:5 v/v) for 24h, and then it was treated with HNO₃: HClO₄ mixture (5:1 v/v). Cadmium concentration in the digest was measured by atomic absorption spectrophotometer Perkin-Elmer (Germany).

Statistical analysis

The experiments were repeated three times with three replications for each repetition. Error bars represent standard errors and each data in figure represents the mean \pm se of at least three independent experiments. The data were processed statistically using the OriginLab 8.5 software statistics package (Northampton, USA).

RESULTS

Growth response

The toxic effect of Cd in maize plants is manifested by an inhibition of both shoots and roots growth. Roots in Cd-treated plants undergo visible alterations, such as root thickening, no lateral root development, or browning of the root tissue. Leaf development was also affected. Leaf emergence was delayed and it remained smaller in size, yellowish area on the leaves could be also observed (data not shown).

Shoot fresh weight accumulation decreased with increasing Cd concentration and 75 μ M Cd caused 40% reduction in the values of this parameter. For root fresh weight the reduction was nearly 30%. Shoot and root lengths were also reduced in Cd-treated variants. These symptoms were not observed in plants treated with 100 μ M SNP. The negative effect of higher Cd concentration (75 μ M) was partially alleviated by treatment with SNP. There was no mitigation effect of supply of SNP to 50 μ M Cd- treated plants (Table 1).

Cd treatment under the present experimental conditions did not induce a water deficit since RWC was slightly affected. In control plants RWC was about 90%. The reduction in this parameter was insignificant for the treated plants (Table 1). This could explain that neither of treatments leads to wilting of plants.

Chlorophyll content showed approximately 20% decrease in Cd treated plants. Treatment with SNP alone increased the chlorophyll level by 15%. Simultaneous treatment of maize plants with Cd and SNP led to almost complete restoration of the values of this parameter compared to untreated control (Table 1).

Leaf gas exchange

The growth inhibition of maize plants by Cd treatment was accompanied by a decrease in the rate of photosynthesis. The most marked reduction was observed after treatment of maize plants with 75 μ M Cd, an almost 4-fold decrease as compared with the control. The exogenous application of NO donor (SNP) to higher applied Cd concentration recovered the rate of CO₂ fixation as compared with Cd-treated variant (Fig. 1). The values of stomatal conductance were lower in Cd-treated plants. No significant changes in this parameter were observed when Cd and NO were presented together in the nutrient solution (Fig 1B). Cd treatment resulted in a significant decrease in the rate of transpiration. The reduction in this parameter was offset by SNP in variant treated with 50 μ M Cd and SNP (Fig. 1C). The results showed that, compare with the control, Cd caused a gradual decrease in WUE. The reduction was retarded by the application of SNP simultaneously with 75 μ M Cd (Fig. 1D).

Carboxylating enzymes activity

The activities of both carboxylating enzymes (PEPC and RuBPC) were also affected by Cd treatment. An increase of PEPC activity by 34% was observed in 50 μ M Cd-treated variant, while RuBPC

Table 1. Effects of 14 days cadmium (Cd) and sodium nitroprusside (SNP) treatments on the shoot and root fresh weight (FW) and length, relative water content (RWC), total chlorophyll (Chl) content and electrolyte leakage of the leaves. The corresponding controls were grown in Hoagland solution. Plants were grown in a growth chamber at $22/18^{\circ}$ C day/night temperatures and a 16h/8h light/dark photoperiod, relative humidity between 50% and 60%, and 180 µmol m⁻² s⁻¹ photosynthetic active radiation.

Treatments	Shoot FW [% of control]	Root FW [% of control]	Shoot length [% of control]	Root length [% of control]	RWC [%]	Total Chl [mg.dm ⁻²]	Electrolyte leakage [µS.gFW ⁻¹]
Control	100	100	100	100	90±2	3.72±0.37	30±6
100 µM SNP	99±1	98±1	100±1	97±2	87±3	4.28±0.19	34±8
50 µM Cd	87±2	92±3	83±3	83±3	87±3	3.34±0.36	39±1
75 µM Cd	60±4	71±3	67±2	57±3	86±4	3.15±0.35	71±1
50 μM Cd+ 100 μM SNP	87±2	88±2	88±2	84±2	86±4	3.84±0.38	42±8
75 μM Cd+ 100 μM SNP	85±2	73±3	85±2	82±3	86±4	3.66±0.30	41±8



Figure 1. Gas exchange parameters of maize leaves treated with cadmium (Cd) and sodium nitroprusside (SNP) individually and after their co-application through nutrient solution for 14 days. The corresponding controls were grown in Hoagland solution. Net CO₂ assimilation rate (A); Stomatal conductance (B); Transpiration rate (C); Water use efficiency (D). Plants were grown for 14 days in a growth chamber at 22/18°C day/night temperatures and a 16h/8h light/dark photoperiod, relative humidity between 50% and 60%, and 180 µmol m⁻² s⁻¹ photosynthetic active radiation. I – Control; II - 100 µM SNP; III - 50 µM Cd; IV - 75 µM Cd; V - 50 µM Cd+ SNP; VI -75 µM Cd+ SNP.

activity showed similar values in both Cd concentrations. Treatment of maize plants with SNP alone did not affect the activity of both enzymes. Simultaneous treatment of maize plants with Cd and SNP did not change the activity of RuBPC while it caused a decrease in PEPC activity (Fig. 2A-B).

Fluorescence

The F_{v}/F_{m} ratio is a measure of the maximum quantum efficiency of PS II photochemistry when all the PS II

and 18%, under 50 and 75 μ M Cd stress respectively in comparison to control plants (Fig. 3). SNP in combination with Cd showed small increase in F_{v}/F_{m} , over the plants treated with Cd alone. The effective quantum yield $\Delta F/F_{m}'$ was much more influenced by Cd treatment than F_{v}/F_{m} ratio. $\Delta F/F_{m}'$ decreased significantly by 30% in all Cd treatments and only 50 μ M Cd+SNP recovered to 82% of the control level. Light-induced photoprotection through thermal dissipation of energy or

centres are open. F_v/F_m decreased by 8%



Figure 2. Activity of phosphoenol pyruvate carboxylase (PEPC) (A), and ribulose 1,5 – bisphosphate carboxylase (RuBPC) (B) in 14 days old maize plants subjected to cadmium (Cd) and sodium nitroprusside (SNP) individually and after their co-application through nutrient solution. The corresponding controls were grown in Hoagland solution. I – Control; II - 100 μ M SNP; III - 50 μ M Cd; IV - 75 μ M Cd; V - 50 μ M Cd+ SNP; VI -75 μ M Cd+ SNP.

non-photochemical quenching (NPQ) regulates and protects photosynthesis in environments where light energy absorption exceeds the capacity for light utilization in photosynthesis (Muller at al 2001). It is very typical in stress conditions to find an increase in NPO. NPO of maize plants increased in 50 µM Cd treated plants over the control and restored to the control values when 50 µM Cd and 100 µM SNP were applied simultaneously. However, 75 µM Cd treated plants supplemented with SNP showed further increase by 21.62% as compared to Cd treated plants alone.

Effect of Cd and SNP on electrolyte leakage

Damage to membrane stability due to Cd indussed oxidative stress was investigated by measuring the level of electrolytes. The electrolyte leakage was evaluated to be 30 μ S.gFW⁻¹ in unstressed plants and was affected by Cd (Table 1). The increase exceeded 100% in 75 μ M Cd compared to the control, while in 50 μ M Cd the extent of change was not so great. Simultaneous treatment of maize plants with 75 μ M Cd and SNP led to levels that are comparable to controls.

Changes in Cd accumulation in leaves and root

Cd content in roots and leaf tissues of control plants (in the absence of Cd in the growth medium) was 7.03 and 7.91 mg.kg DW⁻¹, respectively. It increased by 141.4 and 147-fold in root samples from plants treated with 50 and 75 μ M Cd, respectively. In leaf samples the accumulation of Cd also increased



Figure 3. Effect of cadmium (Cd) and sodium nitroprusside (SNP) individually and after their coapplication through nutrient solution for 14 days on photochemical efficiency of PS II F_v/F_m (A), effective quantum yield $\Delta F/F_m'$ (B) and non-photochemical quenching NPQ (C). The corresponding controls were grown in Hoagland solution. I – Control; II - 100 μ M SNP; III - 50 μ M Cd; IV - 75 μ M Cd; V - 50 μ M Cd+ SNP; VI -75 μ M Cd+ SNP.

but was less pronounced than in roots. Simultaneous treatment of maize plants with Cd and SNP retained the high level of Cd in roots. A positive effect of SNP on leaves was observed only in variant treated with 50 μ M Cd (Fig. 4).



Figure 4. Cadmium accumulation in shoots (A) and roots (B) of maize plants treated with cadmium (Cd) and sodium nitroprusside (SNP) individually and after their simultaneous application through nutrient solution for 14 days. The corresponding controls were grown in Hoagland solution. I – Control; II -100 μ M SNP; III - 50 μ M Cd; IV - 75 μ M Cd; V - 50 μ M Cd+ SNP; VI -75 μ M Cd+ SNP.

DISCUSSION

The present study expands our previously reported finding concerning the putative physiological roles of NO in plants and emphasizes the potential ability of NO to enhance plant tolerance to environmental constrains, like Cd toxicity (Tran et al 2013). It has been shown that simultaneous existence of SNP and Cd in the nutrient solution has a protective effect on growth (Table 1), photosynthesis and transpiration (Fig. 1A, C) and diminishes the oxidative damage caused by Cd.

The control of Cd entry into plants may acts as the first step of the defensive system because it exerts its toxic effects after entering the plant. Plants have evolved a series of defensive strategies to cope with Cd toxicity including the exclusion of Cd, vacuolar compartmentalization, and synthesis of phytochelatins and metallotioneins. In the present study, Cd exposure resulted in higher rate of electrolyte leakage. Similar data have been reported for many plant species, including maize plants (Pal et al., 2005; Krantev et al., 2008), pea (Sandalio et al 2001), beans (Chaoui et al 1997), and others. Cd- induced increase in the values of this parameter suggests a negative impact on membrane integrity leading to membrane deterioration.

Maize plants grown for 14 days with 50 and 75 µM Cd exhibited a significant inhibition of growth, as measured by shoot and root fresh weight accumulation and shoot and root length (Table 1). Although maize is considered to be a relatively Cd-tolerant plant (Pál et al 2006), many toxic symptoms may result if the Cd concentration exceeds a critical level. Another reason for the observed plant growth inhibition could be that under our experimental conditions the plants were exposed to Cd at a very early stage of development and for a long period (14 days). The data showed that the chlorophyll content was reduced (Table 1), and the values of stomatal conductance and WUE were lower in Cdtreated plants (Fig. 1B, D). The activity of Rubisco did not show clear expressed influence, but we found some alterations in the activity of PEPC (Fig. 2). The most obvious effect we found in this study is the observation that Cd treatment caused a very high accumulation of Cd in the roots and much smaller in the leaves (Fig. 4). A higher accumulation of Cd in roots as compared to shoots has been reported by other workers (Sandalio et al., 2001; Krantev et al., 2008). The lower Cd translocation to the leaves found in this study could be related at least in part to lower transpiration in treated maize plants (Fig. 1C). The exogenous application of NO donor (SNP) to Cd-treated plants caused an uptake of photosynthesis and transpiration (Fig. 1).

We assumed several suggestions to explain the protective effects of NO on photosynthesis and attributed them to:

- i. NO improved the photochemical reactions of photosynthesis;
- ii. NO helped to restore the cell membranes integrity;
- iii. NO prevented the accumulation of Cd into the leaves;
- iv. NO protected leaf organization during the early stages of leaf development.

The decrease of the assimilatory potential of photosynthesis, as evidenced by a lower intensity of CO, assimilation rate (Fig. 1A) can also be due to reduced electron transport. In the case of Cd treatment this can be a result of harmful effect of reactive oxygen species on the thylakoid membrane composition and function. Geiken et al. (1998) reported that in the presence of high concentrations of Cd PS II oxygen-evolving complex was altered and disassembly of the stacked regions in chloroplasts of stress pea plants can be seen. The ratio $F_{...}$ F_m is often used as a stress indicator and describes the potential yield of the photochemical reactions. We observed a slightly reduced F_v/F_m ratio in Cd

effective treated samples, whereas quantum yield of PS II $\Delta F/F_{m}^{\prime}$ strongly decreased after Cd treatment. Similar results for inhibition of actual quantum efficiency, and chlorophyll fluorescence decrease ratio (Rfd) were reported by Drazkievicz et al (2003) for maize leaf segments treated with cadmium. The reduction of actual quantum efficiency is due to altered electron transport. Xu et al (2005) found that in rice plants a high concentration of 1mM Cd significantly decreased F_v/F_m and Φ_p as well as photochemical and non-photochemical quenching. It suggested a damage of PS II and blockage of some pathway of excitation energy.

Cadmium at a concentration of 50 uM influenced PS II of maize leaves, seen as an increased non-photochemical quenching (Fig. 3C). Most of the decrease in fluorescence with an increased NPQ is attributed to heat dissipation (Horton and Bowyer 1990). According to Krupa et al (1993), increased NPQ in leaves of plants exposed to Cd ions in the early stages of growth is due to heavy metal poisoning of the Calvin cycle, which results in limitation of ATP and NADPH consumption, causing a high pH-gradient and a limited electron transport. SNP mitigated the heavy metal damage to PS II reaction centre – the levels of light energy transformation efficiency of PS II (F_v/F_m) remain high, but the nonphotochemical quenching (NPQ) showed further increase as compared to the 75 µM Cd treated plants alone.

Our data show that the rate of electrolyte leakage in Cd-treated plants was observed to be higher than that of the control. Upon supplementation of Cd-treatment solution with SNP, there

was a recovery in electrolyte leakage. Electrolyte leakage is related to the extent of free radical production in plants. Cadmium has strong affinity towards nitrogen- and sulfur-containing ligands and proteins. It forms bridges within proteins leading to distorted membrane ion channels and leakage of ions (Mishra et al 2006). As an important member of ROS, O⁻² has been shown to directly react with proteins and to disturb electron transport in photosynthesis (Tan et al., 2008). Reduced electrolyte leakage under Cd and SNP application reflects better membrane integrity and tolerance towards oxidative stress. Popova and Tuan (2010) reported that under severe Cd stress and consequently ROS, the NO can interrupt the lipid peroxidation chain, limiting the damage. Application of SNP suppressed the production of ROS and had a beneficial impact on the effective quantum yield of PS II ($\Delta F/F_m'$) and NPQ and as a result increased photosynthetic rate in the samples with higher applied Cd concentration.

The observed protective effect on root growth and development after simultaneous treatment of maize plants with SNP and Cd is probably due to improved potential of roots to absorb water and mineral elements that keeps the leaves in more favorable conditions than the roots. This suggestion is confirmed by the higher rate of water use efficiency in higher applied Cd concentration (Figure 1D). Another support derives from the data that simultaneous treatment of maize plants with SNP and Cd strongly enhanced the metal accumulation in the roots thus preventing its translocation to the leaves (Fig. 4). Wang et al (2013) showed that the addition of 50, 100, 200 µM SNP to

Cd- treated ryegrass seedlings (Lolium perenne L.) significantly decreased the transport of Cd from roots to shoots alleviated and toxicity symptoms caused by Cd. Here we found a strong decrease of Cd content in leaves of SNP-treated plants, while in the roots a higher Cd accumulation was observed. The lower Cd concentrations in NOexposed leaves may have been caused by reduced Cd uptake and/or by reduced Cd translocation. This suggests that NO decreased roots- to- leaves translocation of Cd or causes sequestration of Cd in roots, resulting in low Cd accumulation in leaves

It was reported that simultaneous treatment of pea plants with SNP and Cd exerted a beneficial effect on leaf structure and guard cell morphology. Treatment with the NO donor SNP alongside Cd mitigated Cd-induced abnormalities in leaf development and structure; additionally, it protected guard cell morphology, which could lead to improved leaf functioning (Tran et al 2013) and could be a reason for higher rate of photosynthesis. We could only suggest that such strategy operates in Cdtreated maize plants but this assumption needs further confirmation.

In conclusion, we obtained experimental evidence indicating that exogenous SNP application is involved in alleviation of Cd-induced inhibition of photosynthesis. Co-application of SNP to Cd ions facilitate the maize seedlings to reduce the negative stress effects through adjustment of the rate of electron transport, preventing the development of the cumulative damage in leaves in response to Cd and recovery of the cell membranes integrity.

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