NITRIC OXIDE ALLEVIATES CADMIUM- BUT NOT ARSENIC-INDUCED DAMAGES IN RICE ROOTS.

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Anotation: Nitrogen monoxide (nitric oxide – NO) is a ubiquitous gaseous molecule involved in numerous animal and plant physiological processes, and it is also a mediator of plant development and response to abiotic/biotic stresses. Different environmental stresses rapidly induce NO-production, which, in turn, participates to the regulation of the plant responses. Several researches have highlighted the involvement of NO in the regulation of plant response to toxic elements, including cadmium (Cd) and arsenic (As) pollutants.

Keywords: Arsenic Cadmium Nitric oxide Oryza sativa Peroxynitrite Root development Superoxide anion.

The NO-involvement in plant physiological/metabolic processes is due to its capability to modify numerous proteins, either directly through post-translational mechanisms, such as S-nitrosylation, nitration and nitrosylation, or indirectly by controlling the transcription of genes that encode proteins involved in stress responses. Various reports highlight that NO has an important role in reducing the damages in plant organs due to abiotic stresses by enhancing the activity of antioxidant enzymes. However, its role in the physiological processes depends on its cellular level. Indeed, at very low

levels it functions as a signal molecule, on the contrary at higher levels becomes a stress-inducing molecule.

Cadmium and As soil pollution is of great concern because it prevents plant development by altering primary metabolic functions, by decreasing water and mineral nutrient uptake, and by inducing a general alteration in organ development, mainly in the root-system. Besides, the presence of Cd and/or As in the soil compromises the commercial value of the edible crops, and represents a potential risk to human health. Cadmium is present in the soil mainly as Cd^{2+}. It easily enters in the root cells using the transporters of the essential nutrients, thus competing with them, or through aquaporins. Arsenic is mostly present in the environment in two inorganic forms: arsenite [As(III)], and arsenate [As(V)]. Organic forms are also possible. Arsenate, being an analogue of phosphate, enters the plant cells by the inorganic phosphate transport system. , whereas arsenite uses the aquaporins of NIP subfamily. In the root cells, As(V) is easily reduced to As(III) , with this reaction contributing to increase the cytosolic levels of ROS.

Nitric oxide decreases Cd and As uptake in rice seedlings

In order to investigate the effects of exogenous NO on Cd or As uptake and translocation, the accumulation of the heavy metal and of the metalloid was evaluated in the roots and shoots of the seedlings. Arsenic and Cd were mainly accumulated in the roots, and As, both as As(III) and As(V), was taken up more than Cd (Fig.1 A–B). The treatment with SNP significantly ($P < 0.01$) reduced the accumulation in the roots of both pollutants (Fig.1). The transport of Cd to the shoot was low, and the co-presence of SNP furtherly and significantly ($P < 0.01$) reduced it (Fig.1 A). Even the As was transported to the shoot at very low amounts, independently from the SNP presence (i.e., 6.41, 4.96, 12.06 and 9.11 mg/kg for As(III), As(III) plus SNP, As(V) and As(V) plus SNP, respectively) (Fig.1 B). The evaluation of the translocation factor (TF) and the bioaccumulation factor (BF) of both the elements, taken alone or combined with SNP, showed that the NO donor did not affect the translocation capability of the heavy metal and the metalloid from the root to the shoot, overall highlighting a significant role of NO in the reduction of the uptake of these elements in rice.

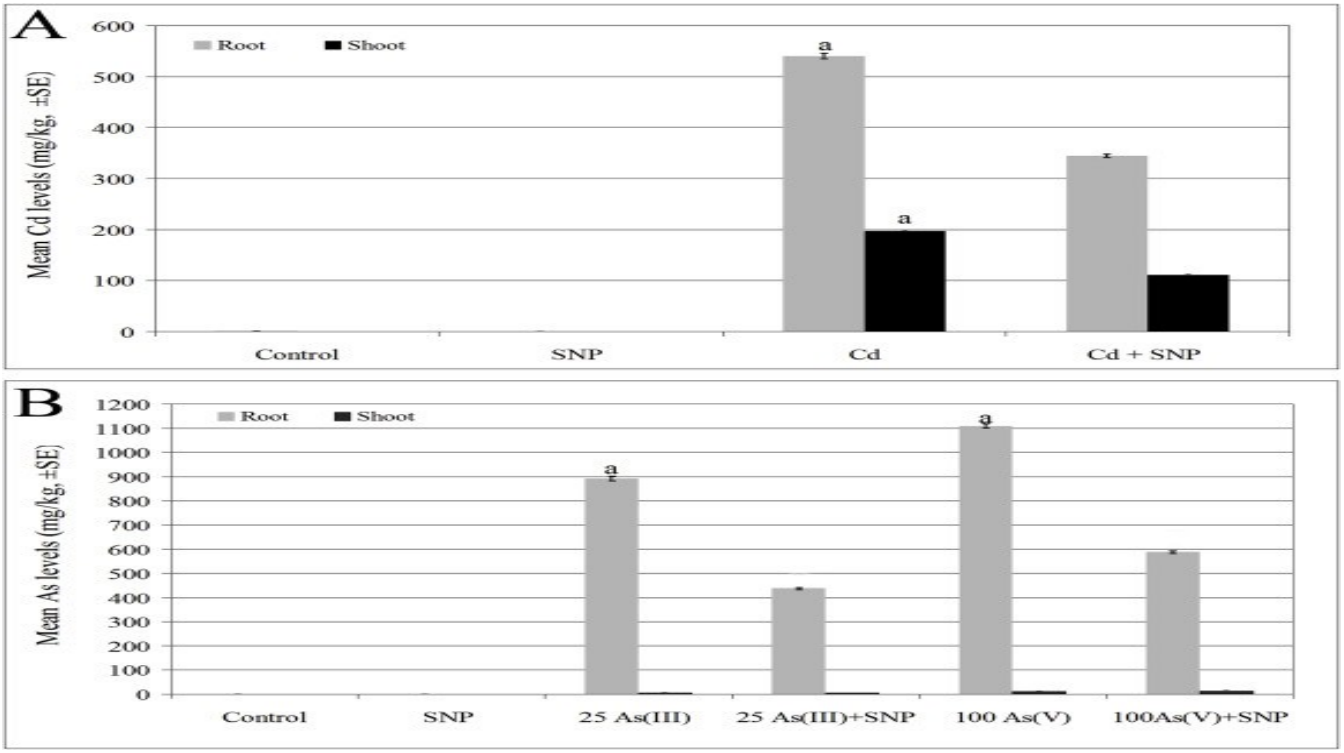

Figure. 1

Cadmium (A) and Arsenic (B) accumulation in roots and shoots of rice seedlings treated or not for 10 days with 100 µM $CdSO_4$ (Cd), 100 µM $Na_2HAsO_4 \cdot 7H_2O$ (As(V)) or 25 µM $NaAsO_2$ (As(III)) or 50 µM $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ (SNP) alone or combined. Letter a shows statistical difference, at least at $P < 0.05$ level, in comparison to the same treatment with/without SNP, for the same organ. Mean of tree biological replicates.

Nitric oxide reduces the histological alterations induced by Cd, but not all those induced by As We deepened the investigation on the role of NO in the root alterations induced by Cd and As through histological and autofluorescence analyses on roots treated or not with Cd or As combined or not with SNP.

Nitric oxide reduces the histological alterations induced by Cd, but not all those induced by As. We deepened the investigation on the role of NO in the root alterations induced by Cd and As through histological and autofluorescence analyses on roots treated or not with Cd or As combined or not with SNP.It is known that Cd and As induce extensive damages in rice AR primary structure during LR-formation. To verify if the increased intracellular NO levels resulted into a reduction in these damages, a histological

analysis was carried out in the AR region forming the LRPs, and the lignification in cell walls detected and quantified (Fig.2).

Fig. 2. Transverse sections of ARs at 2.0 cm from the root tip taken from rice seedlings treated or not for 10 days with 100 µM $CdSO_4$ (Cd), 100 µM $Na_2HAsO_4 \cdot 7H_2O$ (As(V)) alone or combined with 50 µM $Na_2[Fe(CN)_5NO] \cdot 2H_2O$ (SNP). **A**-**B**, **D**-**E**, **G**-**H**, **J**-**K**, **M**-**N** and **P**-**Q** light microscope images of sections stained with toluidine blue. **C**,**F**,**I**, **L**,**O** and **R** images showing lignin autofluorescence (bright blue colour) in sclerenchyma and endodermis cell walls. Bar = 50 µm (B, E, H, K, N, Q) and 100 µm (A, C-D, F-G, I-J, L-M, O–P, R). cp, cortical parenchyma; ep, epidermis; ex, exodermis; s, sclerenchyma layer; en, endodermis. **S**, mean values (±SE) of lignin autofluorescence intensity in sclerenchyma and endodermis measured using ImageJ 1.52a software and expressed in arbitrary units (AUs). Letters a and b show statistical differences, at least at $P < 0.05$ level, for the same tissue and treatment, with/without SNP. Letter c shows statistical differences, at least at $P < 0.05$ level, for the same tissue in comparison with Control. Columns followed by the same letter within the same treatment with/without SNP are not significantly different N = 30.

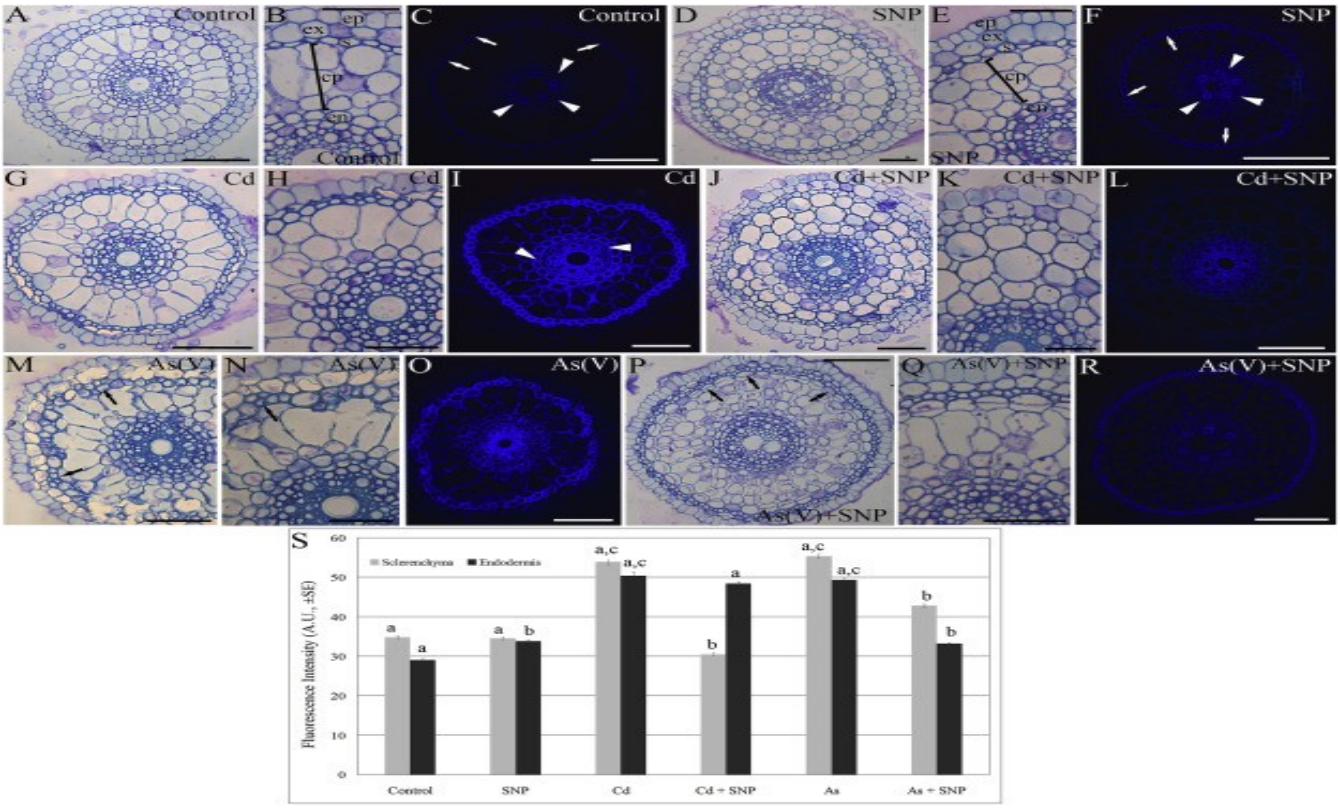

Figure. 2

The histological analysis showed that the SNP-treated roots, were characterized, as the Control ones, by regularly differentiated epidermis, exodermis, sclerenchyma layer, cortical parenchyma, endodermis and vascular bundles (Fig. 2. A–B, D-E). The autofluorescence analysis showed that the sclerenchyma cells were mildly lignified (Fig. 2. C, F, small arrows, and S), while the differentiated endodermis cells did not show regular lignin deposition in the cell walls (Fig. 2. C, F, arrowheads, and S). The Cd-alone-treatment induced a precocious aerenchyma formation and a strong thickening of the sclerenchyma cell walls due to a higher lignin deposition (Fig. 2. G–I and S). Also the endodermic cells were characterized by lignin deposition (Fig. 2. I, arrowheads), and the significant increase of lignin in the cell walls of sclerenchyma and endodermis was also confirmed by the increase of the lignin autofluorescence signal in both tissues (Fig. 2. S). The combined treatment of Cd and SNP significantly reduced cell wall lignification in the sclerenchyma (Fig. 2. J-L), with the lignin autofluorescence signal decreasing up to values comparable to the Control roots Fig. 2. S).

Considering that As(V) and As(III) induced similar histological alterations in root, here we show the images of arsenate-exposed roots only. The As-treatment determined an increase in the sclerenchyma and endodermis cell wall thickening, similarly to Cd, and a related enhancement in lignin autofluorescence (Fig. 2. M–O, S). Moreover, As also induced precocious aerenchyma formation, in addition to an anomalous proliferation of the sclerenchyma cells (Fig. 2. M–N, arrows), and an alteration of the exodermis (Fig. 2. M). The NO-donor combined with As induced the roots to differentiate cells with a reduced lignin deposition, and a reduced autofluorescence signal (Fig. 2. P–R, S), but did not counteract the As-caused anomalous cell proliferation (Fig. 2. P–Q, arrows).

In conclusion, the results highlight that NO differently affects the responses of rice root-system to the toxicity of Cd and As. In fact, increased cellular levels of NO alleviate root damages induced by Cd by improving the entire root-system, but do not improve the root-system ability to counteract As toxicity. The explanation of this different behaviour is probably attributable to the NO-ability to restore the ROS/RNS cellular balance,

differently altered by the two pollutants. Of course, further researches are needed to shed light on the full mechanisms governing NO action in pollutant environments.

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