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Potential Beneficial Effects of Exogenous Nitric Oxide (NO) Application in Plants under Heavy Metal-Induced Stress

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Abstract

Nitric oxide (NO) has become in a key molecule in higher plants because this molecule, direct or indirectly, is involved in a plethora of physiological and (a)biotic stresses. Plants are exposed to many adverse environmental conditions, among them heavy metal contamination has caused unfortunately collateral damage associated with the development of our contemporary lifestyle. Although plants have developed different strategies to palliate the negative effects of these metals, usually there is associated a nitro-oxidative stress. During the last decade, the application of exogenous NO seems to offer some advantages which allows to plants to face the cellular damages induced for heavy metals. Consequently, this update will focus on the potential benefits of the use of NO-donors to counteract the heavy metal harmful effects on plant metabolism.

1. Introduction

Nitric oxide (NO) is a free radical molecule which generates a family of related molecules designated as reactive nitrogen species (RNS) (for a review see Corpas 2016). These molecules play central roles in multiple plant processes from seed germination, development, senescence, fruit ripening and responses to biotic and abiotic stresses (Pagnussat et al., 2002; Besson-Bard et al., 2008; Airaki et al., 2015). These functions seem to be based on the reactivity of NO which can interact either with inorganic and organic molecules including peptides, proteins, lipids, and nucleotides (Corpas et al., 2013; Begara-Morales et al., 2014; Mata-Pérez et al., 2016a,b). Consequently this reactivity explains its many biochemical interactions being a genuine signaling molecule in plants (Simontacchi et al., 2015).

2. Endogenous NO metabolism under heavy metal-induced stress

Heavy metals are chemical elements that share some metallic properties such as ductility, malleability or conductivity and which have high relative atomic weight with an atomic number greater than 20. They can be toxic to plants even at very low concentrations (Corpas et al. 2011; Xiong et al. 2010; Rascio and Navari-Izzo 2011). Heavy metals can also be divided in two categories: i) essential elements required for normal growth and metabolism such as Co, Cu, Fe, Mn, Mo, Ni, and Zn (micronutrients); and ii) non essential elements since they do not make any known physiological function such as Cd, Hg, Se, Pb, or As (this last one is a metalloid but generally is referred to as a heavy metal). In fact, there are many research reports on the different heavy metals showing their toxicity in plants, demonstrating that this toxicity affects in different degrees of seed germination, disturbance of mitosis, inhibition of root and shoot growth, induction of leaf chlorosis, reduction in photosynthesis and DNA synthesis. Furthermore, all these effects are usually accompanied by an overproduction of Reactive Oxygen Species (ROS) which can provoke an oxidative stress (Dixit et al., 2001; Sharma and Dietz, 2009; Palma et al., 2013; Shahid et al., 2014). However, there are accumulating data that RNS metabolism is also affected and consequently the interplay between ROS and RNS can generate the designated nitro-oxidative stress response (Corpas and Barroso, 2013).

Cadmium induced stress could be a good representative model to show how the endogenous NO metabolism could be affected. For example, in pea plants, growth with 50 µM $CdCl_2$ has been reported to provoke a decrease in NO and *S*-nitrosoglutathione (GSNO) content and a lower GSNO reductase activity which was accompanied with a rise of ROS production

Table 1: Examples of beneficial effects of exogenous NO application on plants growth under different heavy metals. APX, ascorbate peroxidase, CAT, catalase. ET, ethylene. JA, jasmonic acid. MDA, malondialdehyde. PCs, phytochelatins. POD, guaiacol peroxidase, SOD, superoxide dismutase, SNP, sodium nitroprusside

Heavy metal	Plant species	Exogenous NO donor	Effects	Reference
Iron				
Fe deficiency	*Zea mays*	100 µM SNP	CAT, POD and APX increased	Sun *et al.*, 2007
Cadmiun				
500 µM Cd^{2+}	*Helianthus annuus*	100 µM SNP	Avoid growth inhibition and chlorophyll degradation. Recovery of CAT activity and GSH levels and enhancement of ASC content and APX activity	Laspina *et al.*, 2005
200 µM Cd^{2+}	*Oryza sativa*	100 µM SNP	Increase pectin and hemicellulose contents in roots	Xiong *et al.*, 2009
25 µM Cd	*Brassica juncea*	5 mM SNP	Reduced the level of proline, non-protein thiols, SOD, APX and CAT	Verma *et al.* 2013
100µM Cd^{2+}	*Oryza sativa*	50 µM SNP	Reduce H_2O_2 and MDA contents. Stimulate the activities of CAT, SOD, APX and POD. Increase accumulation of proline	He *et al.*, 2014
50µM Cd^{2+}	*Oryza sativa*	50 µM SNP	Inhibite Cd-uptake and reversed the Cd-induced toxic effects by restoring membrane integrity	Singh and Shah (2014)
5 mg · l^{-1} Cd^{2+}	*Boehmeria nivea*	100 µM SNP	Increase nitrosothiols and enhance SOD, APX and GR activities. Reduce H_2O_2	Wang *et al.*, 2015
100µM Cd^{2+}	*Trifolium repens*	50 µM SNP	Reduce H_2O_2 and MDA levels. Stimulate activity of antioxidant enzymes. Up-regulate the levels JA and proline. Down-regulate the levels of ET	Liu *et al.*, 2015
Arsenic				
25 or 50 µM AsV	*Oryza sativa*	50 µM SNP	Reduce oxidative damage in the roots	Singh *et al.*, 2009
AsV	*Oryza sativa*	100 µM SNP	Reduce As accumulation and Fe deficiency. Keep GSH/GSSG ratio and reduce PCs content	Singh *et al.*, 2016
AsIII	*Oryza sativa*	30 µM SNP	Reduce As accumulation through down-regulation of *OsLsi1* and *OsLsi2*. Lowered the ROS and MDA content.	Singh *et al.*, 2017
AsiIII	*Oryza sativa*	30 µM SNP	Reduced the Jasmonic acid and As content.	Singh *et al.*, 2017
Copper				
10 mM Cu^{2+}	*Oryza sativa*	100 µM SNP	Reduce Cu-induced toxicity and increase NH^{4+} accumulation	Yu *et al.*, 2005
100 µM Cu^{2+}	*Oryza sativa*	200 µM SNP	Alleviate Cu toxicity	Mostofa *et al.*, 2014
50 µM Cu^{2+}	*Panax ginseng*	100 µM SNP	Increase SOD activity	Tewari *et al.*, 2008
Lead				
100 µM Pb^{2+}	*Arabidopsis thaliana*	100 µM SNP	Reduce the content of H_2O_2 and lipid hydroperoxide	Phang *et al.*, 2011

(Barroso *et al.,* 2006; Rodriguez-Serrano *et al.*, 2006). On the other hand, in tomato plant, under Cd induced stress has shown the interplay between glutathione (GSH) and NO since GSH stimulates simultaneity the accumulation of NO and nitrosothiols as well as the antioxidant capacity improving the tolerance against cadmium stress (Hasan *et al.*, 2016). In the model plant, *Arabidopsis thaliana*, cadmium induces the NO content through NO synthase activity, which contributes to the inhibition of root growth partly caused by iron deprivation (Han *et al.,* 2014). At the subcellular level, Cd stress in *Arabidopsis* has recently been shown to trigger both superoxide radical and NO production in peroxisomes with a concomitant generation of peroxynitrite, supporting the involvement of these organelles in the mechanism of response to this metal (Corpas and Barroso, 2014). More recently, it has been also reported in *Arabidopsis,* the endogenous occurrence of nitro-fatty acids (NO_2-FA), specifically nitro-linolenic acid, which increases under Cd stress, having a signaling function throughout the modulation of transcript levels of heat shock genes encoding heat shock proteins (HSPs) (Mata-Perez *et al.*, 2016b).

3. NO donors and its beneficial effects against heavy metal-induced stress

Research on NO metabolism in animal cells has a long track, with a very relevant significance in the field of biomedicine. Thus, there are many potential applications in areas such as cardiovascular and neurodegenerative diseases, pain and inflammation, dermatology, respiratory disorders and liver diseases. Consequently, there are a significant numbers of chemicals which the capacity to release NO which have been largely used in animal research and for biomedical applications. Among the different NO-donors most commonly employed can be mentioned glyceryl trinitrate, mononitrate isosorbide, diethylamine-NO (DEA-NO, commonly known as NONOates), diazeniumdiolate (NOC-18), sodium nitroprusside (SNP), *S*-nitrosoglutathione (GSNO), *S*-nitroso-N-acetylpenicillamine (SNAP) or 3,3-bis(aminoethyl)-1-hydroxy-2-oxo-1-triazene). However, an important aspect of the application of these chemicals is the dose to be used and the mechanism of NO release which should be carefully considered. Thus, the comparative effects of various NO-donors have indicated that diverse NO redox forms can have different or even opposite effects in animal and plant cells (Wink *et al.*, 1996; Murgia *et al.*, 2004). For example, DEA-NO spontaneously dissociates in a pH-dependent, first-order process with a half-life of 2 min at 37°C and 16 min 22-25°C, pH 7.4, to release 1.5 moles of NO per mole. In the case of SNP which has the formula $Na_2Fe(CN)_5NO$, a concentration of 30 µM SNP (at 37°C) releases NO in a pH dependent manner. Being the greatest quantity of NO released at pH 5.0 with decreasing amounts of NO released up to pH 7.2. However, acidic solutions of SNP exposed to a light source for only a few hours decompose producing a blue smog and an odor of cyanide. Moreover, SNP in aqueous solution is degraded when exposed to white or blue light but no to red light (Grosse and D'Angelo, 2005).

In plant cells, NO research is less developed than that in animal but there is accumulating data indicating that the pretreatment of plants with exogenous NO in different forms and doses can alleviate metal toxicity. In plant research, the most widely used NO donor is the SNP. One of the main reasons could be that this chemical is comparatively cheaper than other NO-releasing chemicals mainly if it is necessary to apply in significant amount to the root system under hydroponic conditions. Table 1 presents several examples of the beneficial effects of the exogenous application of NO in different plant species exposed to several heavy metals and metalloids. In general, it can be inferred that the exogenous NO can reduce the metal accumulation by different mechanisms and attenuate the oxidative damage mainly by the induction of specific antioxidant enzymes.

4. Conclusion

At present, there is no doubt about the relevance of NO metabolism in plant physiology. Although our knowledge about the mechanism of action of NO in plant cells is still in the early stages, it can be confirmed by some potential biotechnological application of NO, since this molecule has been shown to protect and/or alleviate plants against the negative effects of heavy metal-induced stress.

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