

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/278653208>

Function of Nitric Oxide Under Environmental Stress Conditions

Chapter · January 2012

DOI: 10.1007/978-3-642-25829-9_4

CITATIONS

9

READS

78

7 authors, including:



Marina Leterrier

Fermentalg

47 PUBLICATIONS 1,476 CITATIONS

SEE PROFILE



Mounira Chaki

Universidad de Jaén

53 PUBLICATIONS 2,252 CITATIONS

SEE PROFILE



Juan B Barroso

Universidad de Jaén

174 PUBLICATIONS 6,925 CITATIONS

SEE PROFILE



Francisco J Corpas

Spanish National Research Council

247 PUBLICATIONS 11,520 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Function of nitric oxide under physiological and stress conditions [View project](#)



Characterization of antioxidants from pepper fruits [View project](#)

All content following this page was uploaded by [Mounira Chaki](#) on 19 June 2015.

The user has requested enhancement of the downloaded file.

Chapter 4

Function of Nitric Oxide Under Environmental Stress Conditions

Marina Leterrier, Raquel Valderrama, Mounira Chaki, Morak Airaki, José M. Palma, Juan B. Barroso, and Francisco J. Corpas

Abstract Nitric oxide (NO) is a key signaling molecule in different physiological processes of plants. However, under adverse stress conditions, plants can undergo a deregulation in its production which can provoke a process of nitrosative stress. In addition, the exogenous application of NO seems to alleviate or even prevent cellular damage under some specific environmental stresses, suggesting the involvement of this molecule in the mechanism of defense against abiotic stresses. In this article, the current knowledge of the implication of NO under environmental stresses is briefly reviewed with a special emphasis in its interaction with some phytohormones.

4.1 Introduction

Nitric oxide is one of the molecules which have received much attention during the last decade from plant researchers. The main reason is that this free radical is involved as signal molecule in many physiological processes during plant growth and development including seed germination, primary and lateral root growth, flowering, pollen tube growth regulation, fruit ripening, and senescence, among others (Wojtaszek 2000; Corpas et al. 2001, 2004; Lamattina et al. 2003; Magalhaes et al. 2005; Shapiro 2005; Besson-Bard et al. 2008a, b), as well under different environmental stress conditions including heavy metal, salinity, wounding, extreme temperature, etc. (Corpas et al. 2011). However, the real significance of NO and

M. Leterrier • M. Chaki • M. Airaki • J.M. Palma • F.J. Corpas (✉)
Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín (EEZ), CSIC, Granada, Spain
e-mail: javier.corpas@eez.csic.es

R. Valderrama • J.B. Barroso
Grupo de Señalización Molecular y Sistemas Antioxidantes en Plantas, Unidad Asociada al CSIC (EEZ), Área de Bioquímica y Biología Molecular, Universidad de Jaén, Jaen, Spain

related molecules designated as reactive nitrogen species (RNS) is only at the beginning of this attractive research area of plant physiology since many aspects of its biochemistry and physiology are still to be elucidated. Here, it will give a general overview of the basic biochemistry of NO and its interaction with some classical phytohormones. Then, it will summarize briefly the implication of NO under environmental stress conditions taking in consideration that NO depending of its cellular concentration could be a plant regulator (low concentration) or be part of the mechanism of defense as toxic molecule (high concentration).

4.2 Basic Biochemistry of NO

Nitric oxide is a free radical because the nitrogen has an unpaired electron in its π orbital (NO) which determinates its biochemistry. Moreover, NO has a family of related molecules designated as reactive nitrogen species (RNS). Table 4.1 summarizes the main RNS including radical and nonradical molecules. Among the different RNS, peroxyxynitrite (ONOO^-) which is produced by the reaction between NO and superoxide radical ($\text{O}_2^{\cdot-}$) has a relevant significance because it is a powerful oxidant that can mediate nitration process and provokes cellular injury (Szabó et al. 2007; Corpas et al. 2009a; Arasimowicz-Jelonek and Floryszak-Wieczorek 2011).

Nitric oxide can interact with different biomolecules including lipids, nucleic acids, and proteins affecting its functions. However, the interaction with proteins has been the most studied. In this sense, NO directly or indirectly can react with proteins in different ways: (1) with transition metals present in the protein to give complexes called metal nitrosyls, (2) with sulfhydryl groups to render a process of S-nitrosylation, and (3) by adding a nitro ($-\text{NO}_2$) group in a process of nitration. So far, the analysis of NO binding to plant metal-containing protein has been done mainly with plant hemoglobins (Besson-Bard et al. 2008a, b); however, there are some experimental data showing that certain enzyme activities such as cytochrome *c* oxidase, catalase, or ascorbate peroxidase can be modulated by this mechanism (Millar and Day 1996; Clark et al. 2000). Protein S-nitrosylation is a posttranslational modification of cysteine residues produced by NO which can modify the

Table 4.1 Reactive nitrogen species (RNS) including radicals and nonradicals molecules

Radicals	Nonradicals
Nitric oxide (NO)	Nitroxyl anion (NO^-)
Nitrogen dioxide (NO_2)	Nitrosonium cation (NO^+)
	Nitrous acid (HNO_2)
	Dinitrogen trioxide (N_2O_3)
	Dinitrogen tetroxide (N_2O_4)
	Peroxyxynitrite (ONOO^-)
	Peroxyxynitrous acid (ONOOH)
	Alkyl peroxyxynitrite (RNOONO)

function of a broad spectrum of proteins (Stamler et al. 2001; Lindermayr et al. 2005; Wang et al. 2006; Lindermayr and Durner 2009). Special attention must be given in the process of *S*-nitrosylation of the tripeptide glutathione (GSH) to form the *S*-nitrosoglutathione (GSNO) since this molecule can function as mobile reservoir of NO (Durner and Klessig 1999; Barroso et al. 2006) and it can regulate the equilibrium between GSNO and *S*-nitrosylated proteins by a process of transnitrosylation. In this sense, the enzyme GSNO reductase seems to be a key element because it catalyzes the NADH-dependent reduction of (GSNO) to GSSG and NH₃. Consequently, this enzyme controls the intracellular level of GSNO and, as a result, the effects of NO in cells (Letierrier et al. 2011). Protein nitration is another process that introduces a nitro group, (–NO₂) and there are several amino acids which are preferentially nitrated, such as tyrosine(Y), tryptophan (W), cysteine (C), and methionine (M). However, in plants, most studies are focused in tyrosine nitration (Corpas et al. 2009a, b; Chaki et al. 2009). Figure 4.1 shows a straightforward model of NO metabolism in plant cells under environmental stress conditions.

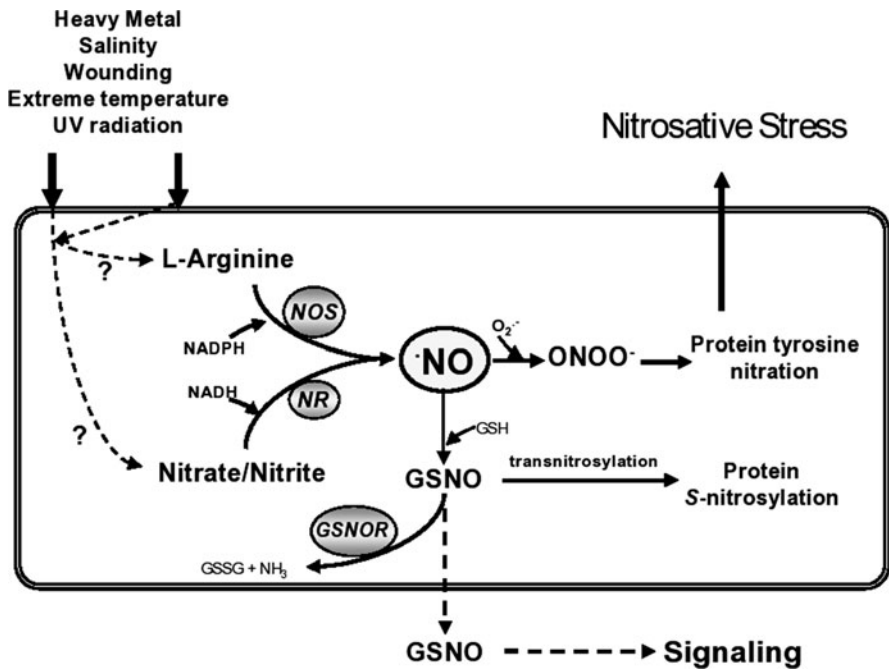


Fig. 4.1 Schematic model of nitric oxide (NO) metabolism in plant cells under environment stress conditions. Under a specific environmental stress, L-arginine-dependent nitric oxide synthase (NOS) and/or nitrate reductase (NR) can generate NO which can react with reduced glutathione (GSH) in the presence of O₂ to form *S*-nitrosoglutathione (GSNO). This metabolite can be converted by the enzyme GSNO reductase (GSNOR) into oxidized glutathione (GSSG) and NH₃. Alternatively, GSNO by a process of transnitrosylation can mediate protein *S*-nitrosylation or diffuse out to the cell where it can act a signal molecule with adjacent cells. On the other hand, NO can react with superoxide radicals (O₂⁻) to generate peroxynitrite (ONOO⁻), a powerful oxidant molecule that can mediate the rise of protein tyrosine nitration which can be considered as a marker of nitrosative stress

4.3 Nitric Oxide: A New Hormone?

Independent of the name, plant hormones, phytohormones, or plant growth regulators are molecules produced inside of plant cells that at low concentrations, in the range nM to pM, promote and influence the growth, development, and differentiation of cells and tissues (Davies 1995; Beligni and Lamattina 2001). Thus, the biosynthesis of plant hormones within plant tissues is often diffuse and not always localized. Classically, there are five groups of plant hormones including auxin (indole-3-acetic acid), cytokinin, gibberellins, ethylene, and abscisic acid (ABA). However, there are also other compounds that have important growth-regulating activities including jasmonic acid (JA), salicylic acid (SA), and brassinosteroids which are considered to function as phytohormones. In this framework, NO could be considered a new member of this group of molecules. However, even when arguments in both directions can be presented, the relevance of NO in physiological processes such as germination, growth, senescence, photosynthesis, stomatal movement, etc. is clear, even though more research must be done in this direction.

4.4 Cross Talk Between NO and Other Hormones Under Stress Conditions

Such as it has been mentioned, NO is implied in numerous plant functions which are also regulated by others phytohormones. So, interactions between NO and other hormones through synergy or antagonism permit a finest level of control depending on the situation of environmental stress.

4.4.1 *Abscisic Acid*

One of the main roles of ABA is to regulate plant response to drought by inducing stomatal closure, therefore reducing water loss via transpiration (Davies and Zhang 1991; Zhu 2002). It is well known that NO is involved in ABA-induced stomatal closure (Garcia-Mata and Lamattina 2002; Desikan et al. 2004; Bright et al. 2006). Moreover, cross talk between ABA and NO also enhances activities of antioxidant enzymes such as catalase and superoxide dismutase. Thus, ABA induces H₂O₂ accumulation, leading to NO generation which in turn activates mitogen-activated protein kinase (MAPK) and upregulates gene expression of antioxidant enzymes (Zhang et al. 2007; Lu et al. 2009). Recently, it has also been shown that cGMP acts downstream of H₂O₂ and NO in the signaling pathway by which ABA induces stomatal closure (Dubovskaya et al. 2011).

NO is also part of the signaling cascade mediating the brassinosteroid-induced ABA biosynthesis in maize leaves under water stress (Zhang et al. 2011b). In maize leaves under UV-B, stress induces ABA production which triggers NO synthesis (Tossi et al. 2009). So, there are some clear interactions between ABA and NO, but the exact nature of this interaction appears to depend on the system studied (plant species and stress).

4.4.2 Ethylene

Ethylene is involved not only in plant growth and senescence but also in plant response to abiotic stress such as salinity, iron deficiency, ozone, and UV-B (Romera and Alcantara 1994; Mackerness et al. 1999; Romera et al. 1999; Vahala et al. 2003; Cao et al. 2007; Ahlfors et al. 2009; Wang et al. 2009).

NO is known to have a protective effect on salt stress (Zhao et al. 2004; Zhang et al. 2006). In fact, in *Arabidopsis* callus under 100 mM NaCl, NO accumulation greatly stimulates ethylene emission, which in turn induces expression of the plasma membrane H⁺-ATPase genes (Wang et al. 2009). The higher activity of the plasma membrane H⁺-ATPase modulates ion homeostasis for a better salt tolerance. Under salt stress, NO-induced ethylene also stimulates the alternative respiratory pathway (Wang et al. 2010a, b).

Ethylene and NO also cooperate in iron homeostasis as it was determined that Fe-related genes upregulated by ethylene were also responsive to nitric oxide (García et al. 2010). Moreover, under iron deficiency, NO induces expression of genes involved in ethylene synthesis, and conversely, ethylene enhances NO production. Hence, both NO and ethylene are necessary for the upregulation of Fe-acquisition genes, and each one influences the production of the other (García et al. 2011). Ethylene and NO also cooperate in ozone stress: both NO- and ethylene-dependent pathways are required for O₃-induced upregulation of alternative oxidase (AOX) in ozone-treated tobacco plants (Ederli et al. 2006). In *Vicia faba*, ethylene participates to UV-B-induced stomatal closure and acts upstream of NO generation (He et al. 2011a, b).

4.4.3 Salicylic Acid

Salicylic acid (SA) induces tolerance to various abiotic stresses such as salinity, heavy metals, and drought (through regulation of stomatal closure) (Manthe et al. 1992; Metwally et al. 2003; Horvath et al. 2007; Szepesi et al. 2009).

NO is involved in the mechanism of salt tolerance generated by SA pretreatment in tomato. Thus, salt stress increases NO content in tomato roots, but pretreatment of the plants with SA changes that response and prevents NO accumulation (Gémes et al. 2011). In *Arabidopsis* roots, SA triggers the NO production through

NOS-dependent pathways where calcium and protein phosphorylation are essential components (Zottini et al. 2007).

SA and NO are both known to reduce heavy metal toxicity separately (Metwally et al. 2003; Arasimowicz and Floryszak-Wieczorek 2007). However, a synergic effect occurs when exogenous applications of both products are combined in canola plants under nickel stress (Kazemi et al. 2010). SA can also induce stomatal closure (Manthe et al. 1992). NO and ROS are both required in SA-induced stomatal closure; in consequence, a model has been proposed where SA activates a peroxidase (sensitive to the inhibitor salicylhydroxamic acid) to produce extracellular ROS, leading to ROS accumulation and NO production in guard cells, and inactivates K_{in}^{+} channels, causing stomatal closure (Khokon et al. 2011).

4.5 Nitric Oxide and Abiotic Stresses

Such it has been mentioned, under stress conditions, there are many reports demonstrating the participation of different phytohormones. For example, under wounding, the participation of several hormones including abscisic acid, ethylene, and jasmonic acid is well recognized (Stratmann 2003), but they are also involved under other stresses such as cadmium or aluminum stress where the production of jasmonic acid, ethylene, gibberellins (GA), or ABA is affected (Sanità di Toppi and Gabbrielli 1999; Rodríguez-Serrano et al. 2009; He et al. 2011a, b). In this context, there are also accumulating data showing the participation of NO in different types of environmental stresses.

4.5.1 Heavy Metal

In plant biology, the term heavy metal is used to design a series of metals and also metalloids that can be toxic to plants even at very low concentrations being for that reason these phytotoxic elements (Corpas et al. 2010; Xiong et al. 2010; Rascio and Navari-Izzo 2011). Thus, heavy metals can also distinguish two categories: (1) essential elements required for normal growth and metabolism such as Co, Cu, Fe, Mn, Mo, Ni, and Zn (micronutrients); and (2) not essential element since they do not perform any known physiological such as Cd, Hg, Se, Pb, or As. Although during the last 10 years, certain numbers of studies have been done in how NO could be involved in the mechanism of response against heavy metals. It must be mentioned that the basic biochemical mechanism is very rudimentary.

4.5.1.1 Cadmium

This is one of the most commonly found heavy metal in soil (Arasimowicz-Jelonek et al. 2011). There are some reports showing the participation of NO under

cadmium stress. For example, in roots of *Pisum sativum* and *Brassica juncea* in the presence of 100 μM Cd, there was a time-dependent endogenous NO production (Bartha et al. 2005). These data contrast with the results observed in leaves and roots of *P. sativum* grown with 50 μM CdCl₂, which produced growth inhibition and oxidative damages (Romero-Puertas et al. 2004), and a drastic reduction of the NO content (Barroso et al. 2006; Rodríguez-Serrano et al. 2006). In addition, the lower NO content in leaves was accompanied by a reduction of GSH, GSNO, and GSNO reductase activity and transcript (Barroso et al. 2006). In contrast, pre-treatment of sunflower seedlings with 100 μM sodium nitroprusside (SNP) protected leaves against Cd-induced oxidative stress (Laspina et al. 2005). In *Lupinus* roots grown with 50 μM Cd, a similar behavior has been observed, and it was proposed that the protective effect of NO could involve the stimulation of superoxide dismutase activity to counteract the overproduction of superoxide radicals (Kopyra and Gwózdź 2003). In the case of rice (*Oryza sativa*) plantlets subjected to 0.2 mM CdCl₂ and treated with 0.1 mM SNP, the toxicity of Cd is alleviated. Accordingly, the authors proposed that the NO-induced increase of Cd tolerance is produced by a rise in pectin and hemicellulose content in the cell wall of rice roots, and this provoked a diminished distribution of Cd in the soluble fraction of leaves and roots but with the concomitant increases of Cd in the cell walls of roots (Xiong et al. 2009). Additionally, the treatment with SNP induces the antioxidant system including ascorbate, catalase, glutathione reductase, and peroxidase which counteracts the oxidative stress provoked by Cd (Hsu and Kao 2004; Panda et al. 2011).

4.5.1.2 Arsenic

It is a metalloid constituent of a wide range of minerals which has become in an important environmental contaminant that can provoke health problems to humans by its accumulation in food crops or in drinking water (Tripathi et al. 2007; Zhao et al. 2010). Under arsenic stress conditions, plants suffer alteration at different levels including elements' uptake and transport, metabolism, and gene expression (Abercrombie et al. 2008; Verbruggen et al. 2009; Zhao et al. 2009). Accumulating data indicate that metabolism of reactive oxygen species (ROS) can be involved and can cause an oxidative stress (Dwivedi et al. 2010). However, there are only few reports analyzing the NO function under arsenic toxicity in higher plants, and they have been done by the exogenous application of NO with sodium nitroprusside (SNP). Thus, in roots of *O. sativa*, the application of SNP provides resistance against As toxicity and has an ameliorating effect against As-induced stress (Singh et al. 2009). Similar observation has been reported in tall fescue where the application of 100 μM SNP reduced arsenic-induced oxidative damage in leaves (Jin et al. 2010). In Arabidopsis seedlings exposed to 0.5 mM arsenate, a significant reduction in growth parameters such as length of roots affecting its membrane integrity and provoking an increase of lipid oxidation has been observed. These changes were accompanied by an alteration of antioxidative enzymes (catalase and glutathione reductase) and nitric oxide metabolism with a significant increase of

NO content, *S*-nitrosogluthathione reductase (GSNOR) activity, and protein tyrosine nitration, which the concomitant reduction of GH and GSNO content. Thus, in this case, arsenate seems to provoke both oxidative and nitrosative stress being the glutathione reductase and GSNOR activities key components in the mechanism of response (Leterrier et al. 2010).

4.5.1.3 Aluminum

Aluminum (Al^{3+}) is not a heavy metal, but it makes up about 8% of the surface of the earth and is the third most abundant element being a major factor limiting crop growth and yield in acid soils since it inhibits cell division, cell extension, and transport (Ma et al. 2001). Thus, *Hibiscus moscheutos* exposed to 100 μM AlCl_3 induced inhibition of root growth. This is accompanied by inhibition of nitric oxide synthase activity and reduced NO content (Tian et al. 2007). In *Arabidopsis thaliana* roots, the cells of the distal portion of the transition zone emitted large amounts of NO, but the treatment with 90 μM aluminum blocked this NO emission (Illés et al. 2006).

In the same way with that of cadmium and arsenic, the treatment with NO donors seems to alleviate the aluminum toxicity. In *Cassia tora* plants pretreated for 12 h with 0.4 mM SNP and subsequently exposed to 10 μM aluminum treatment for 24 h exhibited a significantly greater root elongation and a decrease in Al^{3+} accumulation in root apices as compared with plants without SNP treatment (Wang and Yang 2005). More recently, it has been also shown that SNP treatment in rice (*O. sativa*) provoked an enhancement of Al tolerance in roots. Thus, the mechanism of tolerance is because NO induced a decrease in the contents of pectin and hemicellulose, an increase in the degree of methylation of pectin, and a decrease in the Al accumulation in root cell walls (Zhang et al. 2011a). In the cases of rye (*Secale cereale* L) and wheat (*Triticum aestivum*), Al treatment provoked an inhibition in root growth that was accompanied by a reduction in gibberellin (GA) content and an increase in the values of IAA/GA and ABA/GA. However, treatment with SNP reversed Al toxicity due to an alteration of endogenous hormones in the roots. Thus, SNP reduced the inhibition of root elongation by increasing GA content and decreasing the values of IAA/GA and IAA/zeatin riboside under Al stress (He et al. 2011a, b).

4.5.2 Wounding

Different types of stresses, for example, herbivores, wind, or rain, can produce mechanical injury in plants. To avoid the potential infection by opportunistic microorganisms in the damage zone, plants respond with a cascade of signal that provokes the induction of numerous genes (Schilmiller and Howe 2005). In this sense, there are also data indicating that NO metabolism is also involved.

For example, in *A. thaliana*, mechanical wounding induced a rapid accumulation of NO that could be involved in jasmonic-acid-associated defense responses and adjustments (Huang et al. 2004). In the case of pea (*P. sativum* L.) seedlings, mechanical wounding in leaves provoked an accumulation of NO content after 4 h, and this was accompanied by an increase in the content of *S*-nitrosothiols (SNOs) and a general induction of nitric oxide synthase (NOS) and GSNO reductase activities, although the pattern of proteins that undergo tyrosine nitration did not appear to be affected (Corpas et al. 2008). In sunflower (*Helianthus annuus*) hypocotyls, mechanical wounding apparently did not affect the NO content, but it triggered the accumulation of SNOs, specifically GSNO, due to a downregulation of GSNOR activity, while protein tyrosine nitration increases. Consequently, a process of nitrosative stress is induced, and *S*-nitrosothiols seem to be a new wound signal in plants (Chaki et al. 2011). In other cases, the application of exogenous NO has been reported to modulate the response against wounding. Thus, in tomato (*Lycopersicon esculentum*) plants, the application of NO donors such as SNP or SNAP inhibited the expression of wound-inducible proteinase inhibitors (Orozco-Cardenas and Ryan 2002).

4.5.3 Salinity

Salinity stress takes place when soluble salts (usually NaCl) are elevated in soil, and this affects plant productivity due to its negative effects on plant growth, ion balance, and water relations (Munns and Tester 2008).

One more time, the application of NO donors has been used as tool to study the involvement of NO in plants exposed to salinity stress. For example, in the calluses of reed (*Phragmites communis*) exposed to 200 mM NaCl, the addition of SNP stimulated the expression of the plasma membrane H⁺-ATPase, indicating that NO serves as a signal-inducing salt resistance by increasing the K⁺-to-Na⁺ ratio (Zhao et al. 2004). Similar results have been reported to be found in maize, where the addition of exogenous NO also booted the salt-stress tolerance by elevating the activities of the proton pump and the Na⁺/H⁺ antiport of the tonoplast (Zhang et al. 2006). An 8-day-old rice (*O. sativa*) plant treated with 1 μM SNP or 10 μM H₂O₂ and then exposed to salinity stress has been shown to present an increased tolerance since it is induced by both antioxidant enzymes and some stress-related genes (Uchida et al. 2002). In the case of orange (*Citrus aurantium* L.) trees, similar behavior has been observed suggesting that the induction of antioxidant enzymes as consequence of SNP pretreatment provided a major resistance to salinity (Tanou et al. 2009).

However, there are also reports indicating that salinity affects the endogenous metabolism of NO. Hence, in olive (*Olea europaea* L.) plants grown under *in vivo* salt stress (200 mM NaCl), biochemical analyses demonstrated a general increase in the production of NO, *S*-nitrosothiols, and protein nitration. These data seem to indicate that salinity induced a nitrosative stress (Valderrama et al. 2007). Similar data have been reported in *A. thaliana* with 100 mM NaCl, where using genetic

strategies, it was reported that peroxisomes are responsible for the NO accumulation observed in the cytosol of root cells under this salinity stress conditions (Corpas et al. 2009b).

4.5.4 Atmospheric Pollutants (Ozone and Ultraviolet Radiation)

Ozone (O₃) layer located in the upper atmosphere is a natural component that protects Earth against potential cellular damage by ultraviolet radiation. However, air pollutants resulted in industrial and vehicle emissions such as hydrocarbons and nitrogen oxides found in the troposphere (the lowest layer of the atmosphere) can produce ozone by photochemical reactions, and this ozone negatively affects plants and animals. In plants, the effects of ozone depend of the concentration and exposure time. Low level of ozone reduces photosynthesis and growth and triggers premature leaf senescence in sensitive plant species and cultivars. On the other hand, high concentration of ozone induces cell death with visible injuries in the leaves.

Thus, the interaction of NO with some phytohormones in response to O₃ treatments has been reported. In Arabidopsis, ozone induces the production of NO which is preceded by an accumulation of salicylic acid and then cell death. Interestingly, the application of exogenous NO increased the levels of ozone-induced ethylene production and leaf injury (Rao and Davis 2001). In addition, the accumulation of NO observed after ozone treatments provoked also the induction of genes involved in salicylic acid biosynthesis in Arabidopsis (Ahlfors et al. 2009) and ethylene in tobacco plants (Ederli et al. 2006).

As mentioned before, the increase of atmospheric pollution by compounds such as chlorofluorocarbon used as refrigerants, propellants (in aerosol applications), and solvents contributes also to the destruction of the O₃ layer located in the upper atmosphere which protects against UV radiation. Consequently, its destruction provokes an increase of UV-B radiation (280–320 nm) which affects plant growth and usually induces oxidative stress (reduced photosynthesis, increased damage to DNA). The involvement of NO has also been studied under UV-B radiation. For example, in maize leaves, the treatment with UV-B induced a rise in the content of NO, H₂O₂, and ABA, being this ABA required for the NO-mediated attenuation of deleterious effect of this stress (Tossi et al. 2009). On the other hand, the application of NO donors in bean seedlings subjected to UV-B radiation reduced the UV-B effect characterized by a decrease in chlorophyll contents and oxidative damage to the thylakoid membrane (Shi et al. 2005).

4.6 Perspectives

Many environmental stresses as excess salts, extreme temperatures, toxic metals, air pollutants, etc. constitute a major limitation to agricultural production. To palliate this negative effect is important to progress and integrate different disciplines

such as plant physiology, plant breeding, biochemistry, genetics, molecular biology, agricultural engineering, among others. Considering that NO is involved in a plethora of plant functions under physiological and stress conditions, basic research in plant NO metabolism can be a new piece to contribute and make progress in this direction. So far, there are some promising experimental data which support the relevance of NO in plants under stress conditions. For example, the application of exogenous NO to plants seems to activate different biochemical pathways that provide some resistance against several types of stresses (salinity, heavy metal, ozone, etc.). Therefore, to elucidate NO metabolism in plants can contribute, in coordination with other disciplines, to establish biotechnological strategies against abiotic stresses, which are responsible for important losses in plant yield and crop productivity.

Acknowledgments This work was supported by ERDF-cofinanced grants from the Ministry of Science and Innovation (ACI2009-0860, BIO2009-12003-C02-01 and BIO2009-12003-C02-02).

References

- Abercrombie JM, Halfhill MD, Ranjan P, Rao MR, Saxton AM, Yuan JS, Stewart CN Jr (2008) Transcriptional responses of *Arabidopsis thaliana* plants to As (V) stress. *BMC Plant Biol* 8:87
- Ahlfors R, Brosche M, Kollist H, Kangasjarvi J (2009) Nitric oxide modulates ozone-induced cell death, hormone biosynthesis and gene expression in *Arabidopsis thaliana*. *Plant J* 58:1–12
- Arasimowicz M, Floryszak-Wieczorek J (2007) Nitric oxide as a bioactive signalling molecule in plant stress responses. *Plant Sci* 172(5):876–887
- Arasimowicz-Jelonek M, Floryszak-Wieczorek J (2011) Understanding the fate of peroxynitrite in plant cells from physiology to pathophysiology. *Phytochemistry* 72(8):681–688
- Arasimowicz-Jelonek M, Floryszak-Wieczorek J, Gwózdź EA (2011) The message of nitric oxide in cadmium challenged plants. *Plant Sci* 181(5):612–620. doi:10.1016/j.plantsci.2011.03.019
- Bartha B, Kolbert Z, Erdei L (2005) Nitric oxide production induced by heavy metals in *Brassica juncea* L. Czern. and *Pisum sativum* L. *Acta Biologica Szegediensis* 49:9–12
- Barroso JB, Corpas FJ, Carreras A, Rodríguez-Serrano M, Esteban FJ, Fernández-Ocaña A, Chaki M, Romero-Puertas MC, Valderrama R, Sandalio LM, del Río LA (2006) Localization of S-nitrosoglutathione and expression of S-nitrosoglutathione reductase in pea plants under cadmium stress. *J Exp Bot* 57:1785–1793
- Beligni MV, Lamattina L (2001) Nitric oxide: a nontraditional regulator of plant growth. *Trends Plant Sci* 6:508–509
- Besson-Bard A, Pugin A, Wendehenne D (2008a) New insights into nitric oxide signaling in plants. *Annu Rev Plant Biol* 59:21–39
- Besson-Bard A, Courtois C, Gauthier A, Dahan J, Dobrowolska G, Jeandroz S, Pugin A, Wendehenne D (2008b) Nitric oxide in plants: production and cross-talk with Ca²⁺ signalling. *Mol Plant* 1:218–228
- Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ (2006) ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H₂O₂ synthesis. *Plant J* 45:113–122
- Cao WH, Liu J, He XJ, Mu RL, Zhou HL, Chen SY, Zhang JS (2007) Modulation of ethylene responses affects plant salt-stress responses. *Plant Physiol* 143:707–719
- Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, López-Jaramillo J, Luque F, Palma JM, Pedrajas JR, Begara-Morales JC, Sánchez-Calvo B, Gómez-Rodríguez MV, Corpas FJ, Barroso

- JB (2009) Protein targets of tyrosine nitration in sunflower (*Helianthus annuus* L.) hypocotyls. *J Exp Bot* 60:4221–4234
- Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, Gómez-Rodríguez MV, Pedradas JR, Begara-Morales JC, Sánchez-Calvo B, Luque F, Leterrier M, Corpas FJ, Barroso JB (2011) Mechanical wounding induces a nitrosative stress by downregulation of GSNO reductase and a rise of *S*-nitrosothiols in sunflower (*Helianthus annuus*) seedlings. *J Exp Bot* 62:1803–1813
- Clark D, Durner J, Navarre DA, Klessig DF (2000) Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. *Mol Plant Microbe Interact* 13:1380–1384
- Corpas FJ, Barroso JB, del Río LA (2001) Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. *Trends Plant Sci* 6:145–150
- Corpas FJ, Barroso JB, Carreras A, Quirós M, León AM, Romero-Puertas MC, Esteban FJ, Valderrama R, Palma JM, Sandalio LM, Gómez M, del Río LA (2004) Cellular and subcellular localization of endogenous nitric oxide in young and senescent pea plants. *Plant Physiol* 136:2722–2733
- Corpas FJ, Chaki M, Fernández-Ocaña A, Valderrama R, Palma JM, Carreras A, Begara-Morales JC, Airaki M, del Río LA, Barroso JB (2008) Metabolism of reactive nitrogen species in pea plants under abiotic stress conditions. *Plant Cell Physiol* 49:1711–1722
- Corpas FJ, Chaki M, Leterrier M, Barroso JB (2009a) Protein tyrosine nitration: a new challenge in plants. *Plant Signal Behav* 4:920–923
- Corpas FJ, Hayashi M, Mano S, Nishimura M, Barroso JB (2009b) Peroxisomes are required for *in vivo* nitric oxide accumulation in the cytosol following salinity stress of Arabidopsis plants. *Plant Physiol* 151(4):2083–2094
- Corpas FJ, Palma JM, Leterrier M, del Río LA, Barroso JB (2010) Nitric oxide and abiotic stress in higher plants. In: Hayat S, Mori M, Pichtel J, Ahmad A (eds) *Nitric oxide in plant physiology*. Wiley-VCH, Germany, pp 51–63. ISBN 978-3-527-32519-1
- Corpas FJ, Leterrier M, Valderrama R, Airaki M, Chaki M, Palma JM, Barroso JB (2011) Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. *Plant Sci* 181:604–611
- Davies PJ (1995) The plant hormone concept: concentration, sensitivity and transport. In: Davies PJ (ed) *Plant hormones: physiology, biochemistry and molecular biology*. Kluwer, Dordrecht, pp 13–18
- Davies W, Zhang J (1991) Root signals and the regulation of growth and development of plants in drying soil. *Annu Rev Plant Physiol Plant Mol Biol* 42:55–76
- Desikan R, Cheung MK, Bright J, Henson D, Hancock JT, Neill SJ (2004) ABA, hydrogen peroxide and nitric oxide signalling in stomatal guard cells. *J Exp Bot* 55:205–212
- Dubovskaya LV, Bakakina YS, Kolesneva EV, Sodel DL, McAinsh MR, Hetherington AM, Volotovskii ID (2011) cGMP-dependent ABA-induced stomatal closure in the ABA-insensitive Arabidopsis mutant *abil-1*. *New Phytol* 191(1):57–69
- Durner J, Klessig DF (1999) Nitric oxide as a signal in plants. *Curr Opin Plant Biol* 2:369–374
- Dwivedi S, Tripathi RD, Tripathi P, Kumar A, Dave R, Mishra S, Singh R, Sharma D, Rai UN, Chakrabarty D, Trivedi PK, Adhikari B, Bag MK, Dhankher OP, Tuli R (2010) Arsenate exposure affects amino acids, mineral nutrient status and antioxidants in rice (*Oryza sativa* L.) genotypes. *Environ Sci Technol* 44:9542–9549
- Ederli L, Morettini R, Borgogni A, Wasternack C, Miersch O, Reale L, Ferranti F, Tosti N, Pasqualini S (2006) Interaction between nitric oxide and ethylene in the induction of alternative oxidase in ozone-treated tobacco plants. *Plant Physiol* 142:595–608
- García MJ, Lucena C, Romera FJ, Alcántara E, Pérez-Vicente R (2010) Ethylene and nitric oxide involvement in the up-regulation of key genes related to iron acquisition and homeostasis in Arabidopsis. *J Exp Bot* 61:3885–3899
- García MJ, Suárez V, Romera FJ, Alcántara E, Pérez-Vicente R (2011) A new model involving ethylene, nitric oxide and Fe to explain the regulation of Fe-acquisition genes in strategy I plants. *Plant Physiol Biochem* 49:537–544

- Garcia-Mata C, Lamattina L (2002) Nitric oxide and abscisic acid cross talk in guard cells. *Plant Physiol* 128:790–792
- Gémes K, Poór P, Horváth E, Kolbert Z, Szopkó D, Szepesi A, Tari I (2011) Cross-talk between salicylic acid and NaCl-generated reactive oxygen species and nitric oxide in tomato during acclimation to high salinity. *Physiol Plant* 142(2):179–192
- He JM, Zhang Z, Wang RB, Chen YP (2011b) UV-B-induced stomatal closure occurs via ethylene-dependent NO generation in *Vicia faba*. *Funct Plant Biol* 38:293–302
- Horvath E, Szalai G, Janda T (2007) Induction of abiotic stress tolerance by salicylic acid signaling. *J Plant Growth Regul* 26:290–300
- Hsu YT, Kao CH (2004) Cadmium toxicity is reduced by nitric oxide in rice leaves. *J Plant Growth Regul* 42:227–238
- Huang X, Stettmaier K, Michel C, Hutzler P, Mueller MJ, Durner J (2004) Nitric oxide is induced by wounding and influences jasmonic acid signaling in *Arabidopsis thaliana*. *Planta* 218:938–946
- Illés P, Schlicht M, Pavlovkin J, Lichtscheidl I, Baluska F, Ovecka M (2006) Aluminium toxicity in plants: internalization of aluminium into cells of the transition zone in *Arabidopsis* root apices related to changes in plasma membrane potential, endosomal behaviour, and nitric oxide production. *J Exp Bot* 57:4201–4213
- Jin JW, Xu YF, Huang YF (2010) Protective effect of nitric oxide against arsenic-induced oxidative damage in tall fescue leaves. *Afr J Biotechnol* 9:1619–1627
- Kazemi N, Khavari-Nejad RA, Fahimi H, Saadatmand S, Nejad-Sattari T (2010) Effects of exogenous salicylic acid and nitric oxide on lipid peroxidation and antioxidant enzyme activities in leaves of *Brassica napus* L. under nickel stress. *Sci Hortic* 126:402–407
- Khokon AR, Okuma E, Hossain MA, Munemasa S, Uraji M, Nakamura Y, Mori IC, Murata Y (2011) Involvement of extracellular oxidative burst in salicylic acid-induced stomatal closure in *Arabidopsis*. *Plant Cell Environ* 34:434–443
- Kopyra M, Gwózdź EA (2003) Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiol Biochem* 41:1011–1017
- Laspina NV, Groppa MD, Tomaro ML, Benavides MP (2005) Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. *Plant Sci* 169:323–330
- Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G (2003) Nitric oxide: the versatility of an extensive signal molecule. *Annu Rev Plant Biol* 54:109–136
- Leterrier M, Airaki M, Barroso JB, Palma JM, del Río LA, Corpas FJ (2010) Arsenic impairs the metabolism of RNS and ROS in *Arabidopsis* plant. In: international symposium on the pathophysiology of reactive oxygen and nitrogen species, Salamanca, Spain, p 220 (ISBN: 978-84-692-9284-6)
- Leterrier M, Chaki M, Airaki M, Valderrama R, Palma JM, Barroso JB, Corpas FJ (2011) Function of *S*-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress. *Plant Signal Behav* 6:789–793
- Lindermayr C, Durner J (2009) *S*-Nitrosylation in plants: pattern and function. *J Proteomics* 73(1): 1–9
- Lindermayr C, Saalbach G, Durner J (2005) Proteomic identification of *S*-nitrosylated proteins in *Arabidopsis*. *Plant Physiol* 137(3):921–930
- Lu S, Su W, Li H, Guo Z (2009) Abscisic acid improves drought tolerance of triploid bermudagrass and involves H₂O₂- and NO-induced antioxidant enzyme activities. *Plant Physiol Biochem* 47(2):132–138
- Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. *Trends Plant Sci* 6:273–278
- Mackerness SAH, Surplus SL, Blake P, John CF, Buchanan-Wollaston V, Jordan BR, Thomas B (1999) Ultraviolet-B-induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signalling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. *Plant Cell Environ* 22:1413–1423

- Magalhaes JR, Singh RN, Passos LP (2005) Nitric oxide signaling in higher plants. Studium Press LLC, Houston, pp 1–347
- Manthe B, Schulz M, Schnabl H (1992) Effects of salicylic acid on growth and stomatal movements of *Vicia faba* L: evidence for salicylic acid metabolization. *J Chem Ecol* 18:1525–1539
- Metwally A, Finkemeier I, Georgi M, Dietz KJ (2003) Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiol* 132:272–281
- Millar AH, Day DA (1996) Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. *FEBS Lett* 398:155–158
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651–681
- Orozco-Cardenas ML, Ryan CA (2002) Nitric oxide negatively modulates wound signaling in tomato plants. *Plant Physiol* 130:487–493
- Panda P, Nath S, Chanu TT, Sharma GD, Panda SK (2011) Cadmium stress-induced oxidative stress and role of nitric oxide in rice (*Oryza sativa* L.). *Acta Physiol Plant* 33:1737–1747
- Rao MV, Davis KR (2001) The physiology of ozone induced cell death. *Planta* 213:682–690
- Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant Sci* 180:169–181
- Rodríguez-Serrano M, Romero-Puertas MC, Zabalza A, Corpas FJ, Gómez M, del Río LA, Sandalio LM (2006) Cadmium effect on the oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of ROS and NO accumulation in vivo. *Plant Cell Environ* 29:1532–1544
- Rodríguez-Serrano M, Romero-Puertas MC, Pazmiño DM, Testillano PS, Risueño MC, del Río LA, Sandalio LM (2009) Cellular response of pea plants to cadmium toxicity: cross talk between reactive oxygen species, nitric oxide, and calcium. *Plant Physiol* 150:229–243
- Romera FJ, Alcantara E (1994) Iron deficiency stress response in cucumber (*Cucumis sativus* L) roots: a possible role for ethylene. *Plant Physiol* 105:1133–1138
- Romera FJ, Alcantara E, de la Guardia M (1999) Ethylene production by Fe-deficient roots and its involvement in the regulation of Fe-deficiency stress responses by strategy I plants. *Ann Bot* 83(1):51–55
- Romero-Puertas MC, Rodríguez-Serrano M, Corpas FJ, Gómez M, del Río LA, Sandalio LM (2004) Cadmium-induced subcellular accumulation of O₂⁻ and H₂O₂ in pea leaves. *Plant Cell Environ* 27:1122–1134
- Sanità di Toppi L, Gabbriellini R (1999) Response to cadmium in higher plants. *Environ Exp Bot* 41:105–130
- Schilmiller AL, Howe GA (2005) Systemic signaling in the wound response. *Curr Opin Plant Biol* 8:369–377
- Shapiro AD (2005) Nitric oxide signaling in plants. *Vitam Horm* 72:339–398
- Shi SY, Wang G, Wang YD, Zhang LG, Zhang LX (2005) Protective effect of nitric oxide against oxidative stress under ultraviolet-B radiation. *Nitric Oxide-Biol Chem* 13:1–9
- Singh HP, Kaur S, Batish DR, Sharma VP, Sharma N, Kohli RK (2009) Nitric oxide alleviates arsenic toxicity by reducing oxidative damage in the roots of *Oryza sativa* (rice). *Nitric Oxide* 20(4):289–297
- Stamler JS, Lamas S, Fang FC (2001) Nitrosylation the prototypic redox based signaling mechanism. *Cell* 106:675–683
- Stratmann JW (2003) Long distance run in the wound response-jasmonic acid is pulling ahead. *Trends Plant Sci* 8:247–250
- Szabó C, Ischiropoulos H, Radi R (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nat Rev Drug Discov* 6(8):662–680
- Szepesi A, Csiszár J, Gémes K, Horváth E, Horváth F, Simon ML, Tari I (2009) Salicylic acid improves acclimation to salt stress by stimulating abscisic aldehyde oxidase activity and abscisic acid accumulation, and increases Na⁺ content in leaves without toxicity symptoms in *Solanum lycopersicum* L. *J Plant Physiol* 166:914–925
- Tanou G, Molassiotis A, Diamantidis G (2009) Hydrogen peroxide- and nitric oxide-induced systemic antioxidant prime-like activity under NaCl-stress and stress-free conditions in citrus plants. *J Plant Physiol* 166:1904–1913

- Tian QY, Sun DH, Zhao MG, Zhang WH (2007) Inhibition of nitric oxide synthase (NOS) underlies aluminum-induced inhibition of root elongation in *Hibiscus moscheutos*. *New Phytol* 174:322–331
- Tossi V, Lamattina L, Cassia R (2009) An increase in the concentration of abscisic acid is critical for nitric oxide-mediated plant adaptive responses to UV-B irradiation. *New Phytol* 181:871–879
- Tripathi RD, Srivastava S, Mishra S, Singh N, Tuli R, Gupta DK, Maathuis FJ (2007) Arsenic hazards: strategies for tolerance and remediation by plants. *Trends Biotechnol* 25(4):158–165
- Uchida A, Jagendorf AT, Hibino T, Takabe T (2002) Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Sci* 163:515–523
- Vahala J, Ruonala R, Keinänen M, Tuominen H, Kangasjärvi J (2003) Ethylene insensitivity modulates ozone-induced cell death in birch. *Plant Physiol* 132(1):185–195
- Valderrama R, Corpas FJ, Carreras A, Fernández-Ocaña A, Chaki M, Luque F, Gómez-Rodríguez MV, Colmenero-Varea P, del Río LA, Barroso JB (2007) Nitrosative stress in plants. *FEBS Lett* 581:453–461
- Verbruggen N, Hermans C, Schat H (2009) Mechanisms to cope with arsenic or cadmium excess in plants. *Curr Opin Plant Biol* 12:364–372
- Wang YS, Yang ZM (2005) Nitric oxide reduces aluminum toxicity by preventing oxidative stress in the roots of *Cassia tora* L. *Plant Cell Physiol* 46:1915–1923
- Wang Y, Yun BW, Kwon E, Hong JK, Yoon J, Loake GJ (2006) S-nitrosylation: an emerging redox-based post-translational modification in plants. *J Exp Bot* 57:1777–1784
- Wang H, Liang X, Wan Q, Wang X, Bi Y (2009) Ethylene and nitric oxide are involved in maintaining ion homeostasis in Arabidopsis callus under salt stress. *Planta* 230(2):293–307
- Wang H, Huang J, Bi Y (2010a) Induction of alternative respiratory pathway involves nitric oxide, hydrogen peroxide and ethylene under salt stress. *Plant Signal Behav* 5:1636–1637
- Wang H, Liang X, Huang J, Zhang D, Lu H, Liu Z, Bi Y (2010b) Involvement of ethylene and hydrogen peroxide in induction of alternative respiratory pathway in salt-treated Arabidopsis calluses. *Plant Cell Physiol* 51(10):1754–1765
- Wojtaszek P (2000) Nitric oxide in plants: to NO or not to NO. *Phytochemistry* 54:1–4
- Xiong J, An L, Lu H, Zhu C (2009) Exogenous nitric oxide enhances cadmium tolerance of rice by increasing pectin and hemicellulose contents in root cell wall. *Planta* 230:755–765
- Xiong J, Fu G, Tao L, Zhu C (2010) Roles of nitric oxide in alleviating heavy metal toxicity in plants. *Arch Biochem Biophys* 497:13–20
- Zhang YY, Wang LL, Liu YL, Zhang Q, Wei QP, Zhang WH (2006) Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton-pump and Na⁺/H⁺ antiport in the tonoplast. *Planta* 224:545–555
- Zhang A, Jiang M, Zhang J, Ding H, Xu S, Hu X, Tan M (2007) Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. *New Phytol* 175:36–50
- Zhang Z, Wang H, Wang X, Bi Y (2011a) Nitric oxide enhances aluminium tolerance by affecting cell wall polysaccharides in rice roots. *Plant Cell Rep* 30(9):1701–1711
- Zhang A, Zhang J, Zhang J, Ye N, Zhang H, Tan M, Jiang M (2011b) Nitric oxide mediates brassinosteroid-induced ABA biosynthesis involved in oxidative stress tolerance in maize leaves. *Plant Cell Physiol* 52:181–192
- Zhao LQ, Zhang F, Guo JK, Yang YL, Li BB, Zhang LX (2004) Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. *Plant Physiol* 134:849–857
- Zhao FJ, Ma JF, Meharg AA, McGrath SP (2009) Arsenic uptake and metabolism in plants. *New Phytol* 181:777–794
- Zhao FJ, McGrath SP, Meharg AA (2010) Arsenic as a food chain contaminant: mechanisms of plant uptake and metabolism and mitigation strategies. *Annu Rev Plant Biol* 61:535–559
- Zhu JK (2002) Salt and drought stress signal transduction in plants. *Annu Rev Plant Biol* 53:247–273
- Zottini M, Costa A, De Michele R, Ruzzene M, Carimi F, Lo Schiavo F (2007) Salicylic acid activates nitric oxide synthesis in Arabidopsis. *J Exp Bot* 58:1397–1405