Journal of Plant Physiology 161 (2004) 777–783

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JOURNAL OF
PLANT PHYSIOLOGY

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# Comparative effects of various nitric oxide donors on ferritin regulation, programmed cell death, and cell redox state in plant cells

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Received 2 July 2003; accepted 4 December 2003

KEYWORDS
*Arabidopsis thaliana*;
Ascorbate peroxidase;
Catalase;
Programmed cell death;
Ferritin;
*Nicotiana tabacum*;
Nitric oxide;
NO-donor;
Reactive Oxygen Species

## Summary

Past studies investigating the regulatory functions of nitric oxide (NO) in plant cells have utilized various NO-donors that release NO in different redox forms, which has lead to problems in the interpretation of data. In the present study, the effects of different NO-donors releasing NO with either $NO^+$ (SNP) or $NO^\bullet$ (SNAP, GSNO, NOC-18) character have been compared in plant cells. In particular, ferritin regulation, programmed cell death, cellular redox state, and ROS-scavenging enzymes in *Arabidopsis thaliana* and *Nicotiana tabacum* cells were examined. The results show that SNP behaves differently than the other NO-donors tested; indeed, SNP induces accumulation of ferritin transcripts in *Arabidopsis*, whereas SNAP inhibits its accumulation. Moreover, among the assortment of donors tested, only SNP caused programmed cell death and suppression of ROS-scavenging systems.
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## Introduction

Nitric oxide (NO) is a signaling molecule involved in the regulation of diverse functions in both animal and plant cells (Schmidt and Walter, 1994; Hentze and Kühn, 1996; Murphy, 1999; Durner and Klessig, 1999; Durner et al., 1999; Klessig et al., 2000; Wendehenne et al., 2001; Bogdan, 2001; Beligni

*Abbreviations:* APX, ascorbate peroxidase; CAT, catalase; GOG, glucose/glucose oxidase; GSNO, S-Nitrosoglutathione; IRE, Iron Responsive Element; IRP, Iron Regulatory Protein; NO, nitric oxide; NOC-18, 3,3bis(Aminoethyl)-1-hydroxy-2-oxo-1-triazene; ROS, Reactive Oxygen Species; SNAP, S-Nitroso-*N*-acetylpenicillamine; SNP, sodiumnitroprusside

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doi:10.1016/j.jplph.2003.12.004

and Lamattina, 2001; Neill et al., 2002). The versatile nature of NO is related to its small size, its ability to diffuse freely across membranes, and its high reactivity with many different compounds, which is amplified by the complex interplay between the three NO redox species $NO^+$, $NO^•$ and $NO^-$ (Wendehenne et al., 2001).

Current knowledge on the role of NO in plants is still limited by the lack of experiments based on genetic manipulation of endogenous NO levels, and experimental strategies still rely almost entirely on the use of NO-donors and NO-scavengers.

Several recent reports about the comparative effects of various NO-donors have indicated that diverse NO redox forms can have different or even opposite effects in animal cells (Wink et al., 1996; Kim and Ponka, 1999, 2000; Wardrop et al., 2000; Cairo and Pietrangelo, 2000). In the present study, we have examined the effects of four widely used NO-donors, namely SNP, NOC-18, SNAP, and GSNO, on the expression of ferritin (Murgia et al., 2002), the induction of programmed cell death, alterations in the cellular redox state, and ROS-scavenging capability, processes reported to be altered in plant cells by the presence of NO (de Pinto et al., 2002; Delledonne et al., 1998). Previous studies have shown that high concentrations of SNP and SNAP have similar effects on inhibition of xylem peroxidase in *Zinnia elegans* (Ferrer and Ros Barceló, 1999) and that low SNP and SNAP concentrations also have similar protective effects on potato leaves exposed to oxidative damage (Beligni and Lamattina, 2002).

The data presented here indicate, however, that SNP acts differently from SNAP, NOC-18, and GSNO. The different or even opposite effects of the various NO-donors described in the present study must be taken into account when interpreting on the effects of NO on plant metabolism. Moreover, the different effects triggered by donors that generate NO in different redox forms, timing and concentrations, can provide information on the molecular mechanisms that are responsible for the observed effects.

## Materials and methods

### Chemicals and stock solutions

NO-donors were purchased from Sigma. Stock solutions (100 mM) were prepared immediately before use: SNP, GSNO, and NOC-18 were dissolved in water, while SNAP was dissolved in DMSO. A 50 mM Fe-citrate stock solution was prepared by mixing equal volumes of 100 mM $FeSO_4$ (dissolved in 0.06 N HCl) and 200 mM Na-citrate and was used immediately.

### Cell culture and viability assays

*Arabidopsis thaliana*, ecotype Landsberg and *Nicotiana tabacum*, cv. Bright Yellow 2 cultured cells were grown, propagated, and treated as reported in Murgia et al. (2002) and de Pinto et al. (2002). Cell viability was measured using Trypan Blue staining as described in de Pinto et al. (1999).

### Northern blots

RNA extraction, gel blotting, preparation of *AtFer1* and *Atr18S* probes, and filter hybridization were performed as described in Murgia et al. (2002).

### Ascorbate and glutathione analysis

Tobacco cells were collected by filtration on Whatman 3MM. Cells (0.5–1 g) were resuspended in two volumes of cold 5% (w/v) meta-phosphoric acid. Ascorbate and glutathione pools were extracted from cell suspension through two cycles of freezing-thawing and centrifugation at 20,000*g* for 15 min at 4 °C. The amount and redox state of the two redox pairs were measured according to Zhang and Kirkham (1996).

### Catalase and ascorbate peroxidase assays

Enzyme extraction from tobacco cells was performed according to de Pinto et al. (2000), while native polyacrylamide gel electrophoresis for APX and catalase (CAT) were performed according to de Pinto et al. (2000) and Chandlee and Scandalios (1983), respectively. The protein content of the extracts was measured using the Biorad protein assay kit with bovine serum albumin as a standard.

### Evaluation of NO production

Quantification of NO release by different NO-donors was performed by the Greiss reaction measuring the amount of nitrite and nitrate generated for the spontaneous transformation of NO to $NO_x$. $NO_2^-$ and $NO_3^-$ were determined 48 h after the addition of NO-donors in PBS buffer pH 7.4, according to Privat et al. (1997).

## Results

### The nitric oxide-donor SNP induces accumulation of Arabidopsis ferritin transcript whereas SNAP inhibits its accumulation

Plant ferritins are mainly regulated at the transcriptional level and iron is a strong inducer of *Arabidopsis AtFer1* transcript accumulation (Fobis-Loisy et al., 1995; Lobreaux et al., 1995; Gaymard et al., 1996; Briat and Lobreaux, 1997). NO, a known regulator of animal ferritin (Cairo and Pietrangelo, 2000), is also implicated in the regulation of plant ferritin, as it mediates the iron-induced *Arabidopsis* ferritin accumulation, as demonstrated by using the NO-donor sodiumnitroprusside (SNP) (Murgia et al., 2002).

Our results confirmed that SNP induced accumulation of ferritin transcript in *Arabidopsis* cell cultures (Fig. 1A). In contrast to these results for SNP, the other NO-donors, S-Nitroso-*N*-acetylpenicillamine (SNAP) (Fig. 1B) and GSNO (nitrosoglutathione) (Fig. 1C), were not able to induce such accumulation. NOC-18 (3,3bis(Aminoethyl)-1-hydroxy-2-oxo-1-triazene), another NO-donor, was also unable to induce ferritin transcript accumulation at concentrations of 1 and 5 mM (data not shown).

Notably, treatments with increasing iron concentrations plus SNP (1 or 5 mM) or SNAP (1 or 5 mM) had opposite effects: SNAP inhibited ferritin accumulation induced by iron, whereas SNP did not (Fig. 2A and B compared with Fig. 1A).

The observed effects of SNP on ferritin were not due to the cyanide group, which can be produced by SNP degradation, or to possible modification of cyanide to thiocyanate induced by cell metabolism (Hou et al., 1999). In fact, potassium-ferricyanide (an analogue of SNP that is unable to release NO), potassium ferrocyanide, and sodium thiocyanate all failed to cause accumulation of ferritin transcripts (Fig. 3).

### Only the NO-donor SNP caused tobacco programmed cell death and suppression of ROS-scavenging systems

As previously reported (de Pinto et al., 2002), the simultaneous treatment of tobacco cells with SNP and the $H_2O_2$-generating system glucose–glucose oxidase (GOG) activated a programmed cell death process that was statistically significant 5 h after the addition of 0.5 mM SNP and increased with time (Table 1). Under these experimental conditions, cell death induced by simultaneous production of NO and $H_2O_2$ is a programmed event and was not a consequence of cell necrosis triggered by an overproduction of ROS, as demonstrated in de Pinto et al. (2002) where cytological and biochemical markers of PCD, such as chromatin condensation, cytoplasm shrinkage, and the effect of transcription inhibitors have been already described. When SNP was substituted by one of the other three NO-donors, no significant effects on cell viability were detected (Table 1). The inabilities of SNAP, GSNO, and NOC-18 to affect cell viability were tested using different concentrations of the NO-donors in a range between 0.05 and 1 mM (data not shown).

Since it has been reported that the cell death induced by the simultaneous production of NO and $H_2O_2$ in tobacco cells is preceded by the suppression of antioxidant systems (de Pinto et al., 2002), the effects of the different NO-donors on ascorbate and glutathione pools and redox state, as well as on the APX and CAT activities, were analyzed.

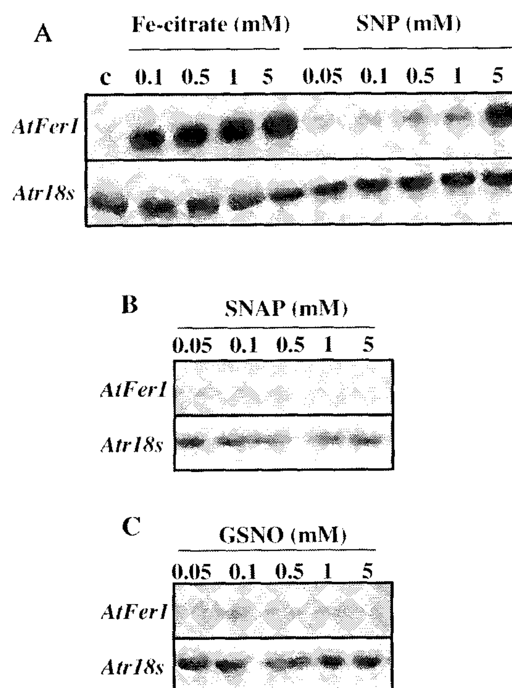


**Figure 1.** Northern blot analysis of *A. thaliana* suspension cultured cells after 3 h treatment with 0.1, 0.5, 1, 5 mM Fe-citrate (A), or increasing concentrations (expressed in mM) of SNP (A), SNAP (B), GSNO (C). c: control (cells with no additions). For (B) and (C) panels, control is the same as in (A). 10 μg total RNA were loaded in each lane. Filters were hybridized with either the ferritin *AtFer1* probe or the constitutively expressed *Atr18s* probe.

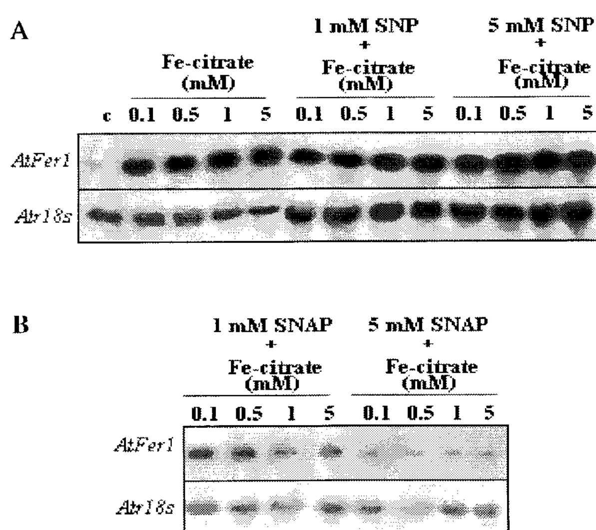


**Figure 2.** Northern blot analysis of *A. thaliana* suspension cultured cells after 3 h treatment with 0.1, 0.5, 1, 5 mM Fe-citrate, 0.1, 0.5, 1, 5 mM Fe-citrate plus: (A) 1 mM or 5 mM SNP; (B) 1 mM or 5 mM SNAP. c: control (cells with no additions). In panel (B) controls are as in (A). 10 μg total RNA were loaded in each lane. Filters were hybridized with either the ferritin *AtFer1* probe or the constitutively expressed *Atr18s* probe.

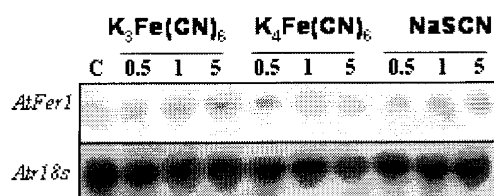


**Figure 3.** Northern blot analysis of *A. thaliana* suspension cultured cells after 3 h treatment with 0.5, 1, 5 mM potassium ferricyanide $K_3Fe(CN)_6$, potassium ferrocyanide $K_4Fe(CN)_6$, or sodium thiocyanate NaSCN. C: controls (cells with no additions). 10 μg total RNA were loaded in each lane. Filters were hybridized with either the ferritin *AtFer1* probe or the constitutively expressed *Atr18s* probe.

The generation in the culture medium of $H_2O_2$ alone (GOG treatments) determined oxidation of the ASC pools, which was evidenced by a decrease in their redox state. Among all of the NO-donors tested, only SNP determined a further shift of ascorbate toward the oxidized form, when generated with $H_2O_2$. This was particularly evident after 7 h of treatment (Table 1, ascorbate redox state column). In contrast, the other NO-donors had a protective effect against the increase in ascorbate oxidation induced by the presence of $H_2O_2$. A similar behavior was also evident for the glutathione pool: only SNP induced an increase in the GSH oxidation as well as a substantial depletion in its total levels (Table 1, glutathione redox state column). The effects of simultaneous generation of NO and $H_2O_2$ on APX and CAT also differed according to the source of NO: only SNP caused the inhibition of both of the ROS-scavenging enzymes, whereas no significant effects were evident for the other NO-donors (Fig. 4).

## Quantification of NO released by different NO donors

To determine if SNAP, GSNO, or NOC-18 affected *Arabidopsis* ferritin expression and the *N. tabacum* antioxidant system differently from SNP because of their reduced efficiency of NO production with respect to SNP, the total amount of NO released by these NO-donors was evaluated indirectly. This was done by measuring the amounts of nitrite and nitrate using the Greiss method (Privat et al., 1997), as their concentrations are a hallmark of past presence of NO and reflect the amount of NO generated by NO-donors (Takahashi et al., 1997).

Nitrite and (nitrite+nitrate) concentrations were measured after 48 h to ensure that the NO produced had been completely transformed to $NO_x$ at the time of measurement, as the method suggests.

The levels of $NO_x$ were higher when generated by SNAP, NOC-18, and GSNO than when generated by SNP (Fig. 5), indicating that the observed phenomena when using SNP were not due to its higher efficiency in producing NO.

## Discussion

In the present study, several effects of various NO-donors on several biological events involving NO in plants have been examined, including regulation of ferritin expression in *A. thaliana* (Murgia et al., 2002), suppression of the ROS-scavenging systems, and induction of programmed cell death in *N. tabacum* (Delledonne et al., 1998; de Pinto et al., 2002). Our results clearly indicate that these processes are differentially regulated when NO is generated by SNP compared to other NO-donors, and in fact the effects of SNP may be opposite to those of SNAP, GSNO, and NOC-18. Only SNP is able to induce the accumulation of ferritin transcripts, cell death, and suppression of antioxidant systems. When NO is generated simultaneously with $H_2O_2$ by using SNP, the shift of the ascorbate and glutathione pools towards the oxidized form is accentuated remarkably in comparison to the shift

**Table 1.** Effects of the NO-donors SNP, SNAP, GSNO, and NOC-18 in combination with $H_2O_2$ on cell viability, ascorbate and glutathione redox pairs in *Nicotiana tabacum* cells, after 5 or 7 h of treatment

| | Treatments | Cell viability (%) | ASC+DHA | Ascorbate redox state | GSH+GSSG | Glutathione redox state |
|---|---|---|---|---|---|---|
| | Control | 99±1 | 678±30 | 0.80±0.03 | 2375±175 | 0.98±0.00 |
| | GOG | 98±1 | 726±35 | 0.67*±0.01 | 2308±145 | 0.96±0.01 |
| | SNP+GOG | 71*±3 | 608*±34 | 0.63*±0.03 | 1420*±130 | 0.86*±0.03 |
| 5 h | SNAP+GOG | 91±2 | 691±51 | 0.84±0.01 | 2209±189 | 0.98±0.01 |
| | GSNO+GOG | 96±2 | 668±58 | 0.85±0.02 | 10218±1005 | 0.91±0.02 |
| | NOC-18+GOG | 94±3 | 628±36 | 0.94*±0.02 | 2344±176 | 0.98±0.01 |
| | Control | 99±1 | 672±60 | 0.80±0.02 | 2076±135 | 0.98±0.00 |
| | GOG | 98±1 | 756±55 | 0.66*±0.01 | 1996±158 | 0.95±0.01 |
| 7 h | SNP+GOG | 32*±3 | 328*±36 | 0.31*±0.03 | 641*±53 | 0.51*±0.03 |
| | SNAP+GOG | 91±2 | 734±32 | 0.80±0.02 | 1649±139 | 0.94±0.02 |
| | GSNO+GOG | 95±2 | 704±38 | 0.81±0.01 | 10276±912 | 0.95±0.02 |
| | NOC-18+GOG | 92±2 | 642±38 | 0.88*±0.02 | 2097±164 | 0.98±0.01 |

Each NO-donor was used at 0.5 mM concentration. Each sample, except the control, was also treated with GOG (0.5 mM glucose plus 0.5 U/ml glucose oxidase). Ascorbate and glutathione pools were measured as nmol per gram fresh weight. Ascorbic acid and glutathione redox states were calculated as ASC/(ASC+DHA) and GSH/(GSH+GSSG), respectively. The results are the means of four independent experiments ±SE.

*Indicates statistically significant differences with respect to the control. In all cases, $P$ was 0.05 using the Student $T$ test.

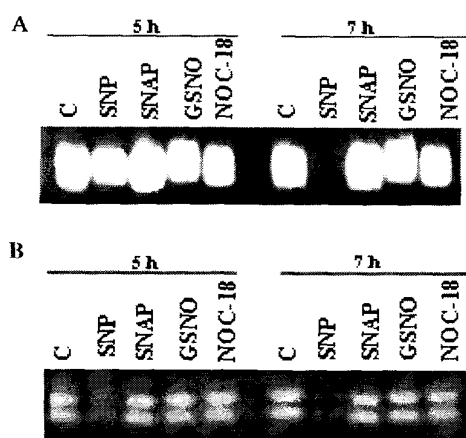


**Figure 4.** (A) CAT activity gel of *N. tabacum* cells treated with SNP, SNAP, NOC-18, or GSNO. All samples, except the control (C), were treated with GOG (0.5 mM glucose plus 0.5 U/ml glucose oxidase) and 0.5 mM NO-donors. Cell sampling was performed after either 5 or 7 h. 80 μg of protein extract were loaded in each lane. (B) APX activity gel of *N. tabacum* cells. Treatments were performed as for CAT. 300 μg of protein extracts were loaded in each lane.

induced by the generation of $H_2O_2$ alone. It has been previously reported that the changes in the ascorbate and glutathione redox balance, as well as the suppression of APX, are part of the signaling pathway that triggers programmed cell death (de Pinto et al., 2002; Mittler et al., 1998, 1999). In contrast, the NO generated by SNAP, NOC-18, or GSNO does not activate programmed cell death. Moreover, these NO-donors completely reverse the oxidation of ascorbate induced by $H_2O_2$.

Notably, the different effects of the various NO-donors on plant cells presented in this work are not due to the inefficacy in generating NO, since SNAP, GSNO, and NOC-18 all generate higher amounts of NO with respect to SNP. Moreover, SNAP, GSNO, and NOC-18 behave differently from SNP on both ferritin expression and cell death induction, even when they are used at concentrations that generate quantities of NO comparable to SNP alone (data not shown).

Differences in the predominant redox form in which NO is released, namely $NO^+$ for SNP and $NO^•$ for SNAP, GSNO, and NOC-18, could at least in part explain the effects of the various NO-donors (Hou et al., 1999; Bogdan, 2001; Kim and Ponka, 1999; Kim and Ponka, 2000).

A molecular explanation for the different effects of $NO^•$ and $NO^+$ on ferritin regulation in murine macrophages has already been proposed (Kim and Ponka, 1999, 2000).

Animal ferritin mRNA contains an iron responsive element (IRE) in its 5′ UTR that constitutes the binding site for the related iron regulatory proteins IRP-1 and IRP-2. IRP-1 is a bifunctional protein that can possess a cubane [4Fe-4S] cluster. When the cluster is present, the protein loses its IRE binding activity and acquires aconitase activity (Hentze and Kühn, 1996; Cairo and Pietrangelo, 2000).

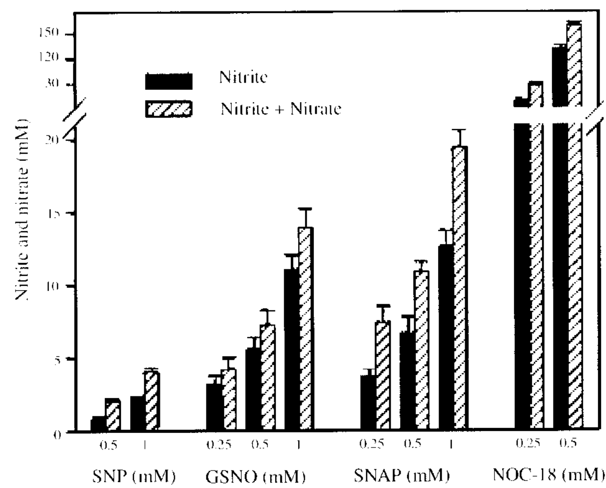


**Figure 5.** The amount of $NO^{2-}$ and $NO^{3-}$ was determined by Greiss reaction after 48 h following the addition of the NO-donors in PBS. The values are the means of four independent experiments ±SE.

The binding of IRP-1/IRP-2 to IRE inhibits the translation of ferritin mRNA (Hentze and Kühn, 1996). NO favors the binding of IRP-1 when present in the radical $NO^{\bullet}$ form and decreases the activity of IRP-1 and IRP-2 when present in the $NO^{+}$ form (Kim and Ponka, 1999, 2000). The current hypothesis is that $NO^{\bullet}$ interacts with the aconitase [4Fe-4S] cluster, thereby causing its disassembly. On the other hand, $NO^{+}$ S-nitrosylates thiol groups in IRP-1 and IRP-2, thus inhibiting their binding to IRE. The S-nitrosylated IRP-2 is then degraded through the ubiquitin-proteasome pathway (Kim and Ponka, 1999, 2000; Cairo and Pietrangelo, 2000).

The *Arabidopsis AtFer1* ferritin promoter possesses an iron dependent regulatory sequence (IDRS) that is responsible for ferritin repression under low iron conditions. However, the proteic factor that binds to the IDRS, inhibiting the transcription of ferritin (Petit et al., 2001), has not yet been identified.

The next challenge will be to isolate this proteic factor and to verify whether it posesses, in analogy to the mammalian IRP-1/IRP-2, a [4Fe-4S] cluster subject to $NO^{\bullet}$ regulation and whether the stability of this unknown factor is differently regulated by the $NO^{\bullet}$ and $NO^{+}$ forms.

The results of the present work regarding the effects of various NO-donors on *Arabidopsis* ferritin were not integrated with experiments on *N. tabacum* cells, as no ferritin genes have been cloned yet in *N. tabacum*.

The different NO-donors could also have diverse effects on various cellular functions because of the dissimilar amounts of NO released over time (Wink et al., 1996). The results shown here, however, do not indicate the amount of NO as the critical point for triggering different metabolic responses.

The hypothesis that different chemical forms of NO can be differently perceived by plant cells raises new questions on NO-dependent cellular signaling, which require further study, especially using different donors that can provide further hints about the mechanism of NO action.

## Acknowledgements

This work was partially supported by the Ministero della Istruzione Università e Ricerca (PRIN). The authors are grateful to Dr. Nicoletta Beffagna (C.N.R., via Celoria 26, Milano) for kindly providing *Arabidopsis* cell cultures.

## References

Beligni MV, Lamattina L. Nitric oxide in plants: the history is just beginning. Plant Cell Env 2001;24: 267 78.

Beligni MV, Lamattina L. Nitric oxide interferes with plant photo-oxidative stress by detoxifying reactive oxygen species. Plant Cell Env 2002;25:737–48.

Bogdan C. Nitric oxide and the regulation of gene expression. Trends Cell Biol 2001;1:66–75.

Briat JF, Lobreaux S. Iron transport and storage in plants. Trends Plant Sci 1997;2:187–92.

Cairo G, Pietrangelo A. Iron regulatory proteins in pathobiology. Biochem J 2000;352:241–50.

Chandlee JM, Scandalios JG. Gene expression during early kernel developmental in *Zea mays*. Dev Genetics 1983;4:99–115.

Delledonne M, Xia Y, Dixon RA, Lamb C. Nitric oxide functions as signal in plant disease resistance. Nature 1998;394:585–8.
de Pinto MC, Francis D, De Gara L. The redox state of the ascorbate-dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. Protoplasma 1999;209:90–7.
de Pinto MC, Tommasi F, De Gara L. Enzymes of the ascorbate biosynthesis and ascorbate-glutathione cycle in cultured cells of tobacco Bright Yellow 2. Plant Physiol Biochem 2000;38:541–50.
de Pinto MC, Tommasi F, De Gara L. Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright Yellow 2 cells. Plant Physiol 2002;130:698–708.
Durner J, Gow AJ, Stamler J, Glazebook J. Ancient origins of nitric oxide signaling in biological systems. Proc Natl Acad Sci USA 1999;96:14206–7.
Durner J, Klessig DF. Nitric oxide as a signal in plants. Curr Opt Plant Biol 1999;2:369–74.
Ferrer MA, Ros Barcelò A. Differential effects of nitric oxide on peroxidase and $H_2O_2$ production by the xylem of *Zinnia elegans*. Plant Cell Envrion 1999;22:891–7.
Fobis-Loisy I, Loridon K, Lebrun M, Briat JF. Structure and differential expression of two maize ferritin genes in response to iron and abscissic acid. Eur J Biochem 1995;231:609–19.
Gaymard F, Boucherez J, Briat JF. Characterization of a ferritin mRNA from *Arabidopsis thaliana* accumulated in response to iron through an oxidative pathway independent of abscissic acid. Biochem J 1996; 318:67–73.
Hentze MW, Kühn LC. Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide and oxidative stress. Proc Natl Acad Sci USA 1996;93:8175–82.
Hou YC, Janczuk A, Wang PG. Current trends in the development of nitric oxide donors. Curr Pharma Design 1999;5:417–41.
Kim S, Ponka P. Control of transferrin receptor expression via nitric-oxide mediated modulation of iron regulatory protein-2. J Biol Chem 1999;274:33035–42.
Kim S, Ponka P. Effects of interferon-$\gamma$ and lipopolysaccharide on macrophage iron metabolism are mediated by nitric oxide induced degradation of iron-regulatory protein-2. J Biol Chem 2000;275:6220–6.
Klessig DF, Durner J, Noad R, Navarre DA, Wendehenne D, Ku Cmar D, Zhou JM, Shah J, Zhang S, Kachroo P, Trifa Y, Pontier D, Lam E, Silva H. Nitric oxide and salicylic acid signaling in plant defence. Proc Natl Acad Sci USA 2000;97:8849–55.
Lobreaux S, Thoiron S, Briat JF. Induction of ferritin synthesis in maize leaves by an iron-mediated oxidative stress. Plant J 1995;8:443–9.
Mittler R, Feng X, Cohen M. Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. Plant Cell 1998;10:461–73.
Mittler R, Herr EH, Orvar BL, Van Camp W, Willekens H, Inzé D, Ellis BE. Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc Natl Acad Sci USA 1999;96: 14165–70.
Murgia I, Delledonne M, Soave C. Nitric oxide mediates iron-induced ferritin accumulation in *Arabidopsis thaliana*. Plant J 2002;30:521–8.
Murphy MP. Nitric oxide and cell death. Biochim Biophys Acta 1999;1411:401–14.
Neill S, Desikan R, Clarke A, Hurst RD, Hancock JT. Hydrogen peroxide and nitric oxide as signalling molecules in plants. J Exp Bot 2002;53:1237–47.
Petit JM, van Wuytswinkel O, Briat JF, Lobreaux S. Characterization of an iron-dependent regulatory sequence involved in the transcriptional control of *AtFer1* and *ZmFer1* plant ferritin genes by iron. J Biol Chem 2001;276:5584–90.
Privat C, Lantoine F, Bedioui F, Millanovoye van Brussel E, Devynck J, Devynck MA. Nitric oxide production by endothelial cells: comparison of three methods of quantification. Life Sci 1997;61:1193–202.
Schmidt HH, Walter U. NO at work. Cell 1994;78:919–25.
Takahashi K, Hara E, Ogawa K, Fujita H, Shibahara S. Possible implications of the induction of human heme oxygenase-1 by nitric oxide donors. J Biochem 1997; 121:1162–8.
Wardrop SL, Watts RN, Richardson DR. Nitrogen monoxide activates Iron Regulatory Protein 1 RNA-binding activity by two possible mechanisms: effect of the [4Se–4S] cluster and iron mobilization from cells. Biochem 2000;39:2748–58.
Wendehenne D, Pugin A, Klessig D, Durner J. Nitric oxide: comparative synthesis and signaling in animal and plant cells. Trends Plant Sci 2001;6:177–83.
Wink DA, Cook JA, Pacelli R, DeGraff W, Gamson J, Liebmann J, Krishna MA, Mitchell JB. The effect of various nitric oxide-donor agents on hydrogen peroxide-mediated toxicity: a direct correlation between nitric oxide formation and protection. Arch Biochem Biophys 1996;331:241–8.
Zhang J, Kirkham MB. Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol 1996; 132:361–74.