Nitric oxide: a new player in plant signalling and defence responses
David Wendehenne¹, Jörg Durner² and Daniel F Klessig³

There is increasing evidence that nitric oxide (NO), which was first identified as a unique diffusible molecular messenger in animals, plays important roles in diverse (patho)physiological processes in plants. NO functions include the modulation of hormonal, wounding and defence responses, as well as the regulation of cell death. Enzymes that catalyse NO synthesis and signalling cascades that mediate NO effects have recently been discovered, providing a better understanding of the mechanisms by which NO influences plant responses to various stimuli. Additionally, growing evidence suggests that NO signalling interacts with the salicylic acid and jasmonic acid signalling pathways.

NO synthesis
In animals, NO is generated primarily by nitric oxide synthase(s) (NOS), a group of evolutionarily conserved cytosolic or membrane-bound isoenzymes that convert l-arginine to l-citrulline and NO[2,8]. In plants, although NOS-like activity has been reported widely, it has not been possible to identify corresponding plant proteins using antibodies against mammalian NOS(s)[9]. Recently, however, two unrelated groups of NOS-like enzymes have been identified in plants: a pathogen-inducible NOS from Arabidopsis thaliana and tobacco (iNOS; [10]) and a hormone-activated NOS from A. thaliana (AtNOS1; [5**]). The pathogen-inducible NOS is a variant of the P protein of the glycine decarboxylase complex (GDC) [10**]. It displays typical NOS activity and requires the same co-factors as its mammalian counterparts (Table 1). However, as only a few of the conserved domains required for NO synthesis by animal/microbial NOS are evident in this enzyme, iNOS probably uses distinct chemistry to generate NO. iNOS was shown to produce NO in A. thaliana plants that were resisting infection by turnip crinkle virus [10**], and in tobacco plants treated with tobacco mosaic virus (TMV) or the fungal elicitor cryptogein [10**,11]. Furthermore, iNOS is a key enzyme for the maintenance of basal resistance to Pseudomonas syringae in tomato [12*]. The hormone-activated NOS was cloned on the basis of its sequence similarity to a protein implicated in NO synthesis in the snail Helix pomatia [5**]. AtNOS1 does not share sequence identity with either mammalian NOS or the plant iNOS, and surprisingly, displays a flavin-, heme- and tetrahydrobiopterin-independent NOS activity (Table 1). AtNOS1 has been implicated in NO production in response to abscisic acid (ABA) [5**]. Moreover, because an AtNOS1 knock-out mutant showed reduced growth and fertility, it is probable that AtNOS1 catalyses NO production in response to a wide range of hormonal and other signals.

Abbreviations
ABA abscisic acid
cADPR cyclic ADP-ribose
GC guanylate cyclase
GDC glycine decarboxylase complex
HR hypersensitive response
iNOS pathogen-inducible NOS
JA jasmonic acid
NO nitric oxide
NOS nitric oxide synthase(s)
NR nitrate reductase
PAL phenylalanine ammonia lyase gene
PCD programmed cell death
PR pathogenesis-related gene
SA salicylic acid
sGC soluble GC
TMV tobacco mosaic virus

Introduction
As recently as 1987, nitric oxide (NO) was widely considered to be just a toxic gas. By the early 1990s, however, overwhelming evidence argued that NO was an integral part of normal physiological processes in animals. This discovery led to a major revolution in biomedical research [1,2]. By the late 1990s, NO was identified as an important messenger in plant defence signalling against microbial pathogens [3,4]; it was subsequently shown to be a crucial player in the regulation of normal plant physiological processes, including stomatal closure, growth and development [5**,6,7**]. In this review, we discuss recent progress that has deepened our understanding of NO synthesis and signalling functions in plants, with special emphasis on hormonal and defence signalling.
Nitrate reductase (NR) is another enzyme that is capable of producing NO in plants [13,14]. It catalyses the NAD(P)H-dependent reduction of nitrite to NO (Table 1). Genetic evidence indicates that NR-mediated NO synthesis is required for ABA signalling in A. thaliana [15], a function also assigned to AtNOS1 [5**]. In addition, because NR gene expression is induced at the transcriptional and protein levels in potato tubers treated with either Phytophthora infestans or an elicitor derived from this oomycete pathogen, NR may synthesise NO during plant defence [16].

NO production may involve not only other currently unidentified enzyme activities but also non-enzymatic principles (Table 1; [17]). For instance, recent work has shown that a non-enzymatic reduction of nitrite to NO occurs in the apoplast of barley aleurone layers [18*]. This NO production required an acidic pH and was accelerated by reducing agents such as phenolic compounds. Furthermore, apoplastic non-enzymatic NO synthesis was observed in response to gibberellin and ABA, two hormones that rapidly acidify the apoplastic medium. The physiological significance of this non-enzymatic NO production is currently unknown.

cGMP, cADPR and Ca^{2+}: three second messengers mediating NO signalling

Soluble guanylate cyclase (sGC) is a crucial component of NO signalling in animals. NO binds to sGC heme, thereby activating the enzyme and increasing the level of the second messenger cGMP. cGMP, in turn, transiently activates various cGMP targets. A similar transduction system appears to function in plants. Treatment of tobacco leaves or suspension cultures with NO induces a transient increase in endogenous cGMP concentrations [4]. Furthermore, sGC inhibitors block NO-induced activation of phenylalanine ammonia lyase gene (PAL) expression and PAL enzyme activity in tobacco [4], as well as NO- and auxin-mediated root development in cucumber [6]. However, major components of the NO/cGMP pathway, including NO-sensitive guanylate cyclase (GC) and the cGMP targets, have yet to be identified.

In addition to cGMP, NO may exert its functions through cyclic ADP-ribose (cADPR) and Ca^{2+} mobilisation. In animal and plant cells, cADPR functions as a second messenger to stimulate Ca^{2+} release through intracellular Ca^{2+}-permeable ryanodine receptor channels (RYR). In animals, NO activates cADPR synthesis via a cGMP-dependent pathway [8]. Growing evidence suggests that a comparable NO-regulated signalling cascade operates in plants. In tobacco, cADPR induced the expression of the PAL and pathogenesis-related (PR)-1 genes through a signalling cascade that is sensitive to RYR inhibitors (Figure 1; [4]), whereas the cADPR antagonist 8-Br-cADPR suppressed the induction of PR-1 expression by NO [19]. Furthermore, NO promoted increased levels of cytosolic Ca^{2+} in Vicia faba guard cells [20*]. In these cells, as in tobacco [4,19], NO appears to act through cGMP and cADPR to activate intracellular Ca^{2+}-permeable channels. NO also plays a role in elevating free cytosolic Ca^{2+} in tobacco cells that are responding to hyperosmotic stress or cryptopine [11,21]. In addition, NO, along with cGMP and cADPR, mediates ABA-induced stomatal closure in guard cells [7**]. Ca^{2+} is also implicated in this process: the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) prevented ABA-induced inactivation of the inward-rectifying K^{+} channel and activation of the outward-rectifying Cl^{-} channel, two Ca^{2+}-dependent events that are essential for stomatal closure (Figure 1; [20*]).

It should be noted that NO affects the expression of numerous plant genes in addition to PAL and PR-1 (Table 2). The NO-dependent intracellular signalling pathway(s) that lead to the activation or suppression of these genes have not yet been defined. As Ca^{2+} appears to

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**Table 1**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Co-factors</th>
<th>Cellular localisation</th>
<th>Physiological process affected</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal NOS</td>
<td>l-Arg</td>
<td>Heme, NADPH, FAD, PMN, H_{2}B, calmodulin</td>
<td>Cytosol, PM bound, chloroplasts</td>
<td>Neuro-transmission, immune response, vasodilatation [2,8]</td>
</tr>
<tr>
<td>Plant iNOS</td>
<td>l-Arg</td>
<td>Heme, NADPH, FAD, H_{2}B, calmodulin</td>
<td>Cytosol, mitochondria</td>
<td>Defence responses to pathogens [10**,11,12]**</td>
</tr>
<tr>
<td>Plant AtNOS1</td>
<td>l-Arg</td>
<td>NADPH, calmodulin</td>
<td>ND</td>
<td>ABA signalling, growth and development, fertility [5**]</td>
</tr>
<tr>
<td>Plant NR</td>
<td>Nitrite</td>
<td>NAD(P)H</td>
<td>Cytosol</td>
<td>ABA signalling, defence responses [13,14,15*,16]</td>
</tr>
<tr>
<td>Plant Ni-NOR</td>
<td>Nitrite</td>
<td>Phenolics, acidic pH</td>
<td>PM bound</td>
<td>ND [39]</td>
</tr>
<tr>
<td>Non-enzymatic NO production</td>
<td>Nitrite</td>
<td>Phenolics, acidic pH</td>
<td>Apoplast</td>
<td>ND [18*]</td>
</tr>
</tbody>
</table>

*For details of the enzymology of animal NOS, the reader is referred to [2]. Abbreviations: H_{2}B, tetrahydrobiopterin; GM, Golgi membrane; ND, not determined; Ni-NOR, a 310-kDa plasma-membrane-bound enzyme that catalyses the reduction of nitrite to NO; PM, plasma membrane; (?) suggested but not demonstrated.
play a central role in mediating NO/redox-sensitive processes in plants, however, the NO-induced regulation of at least some of these genes probably involves the modulation of intracellular Ca\(^{2+}\) levels via the Ca\(^{2+}\) modifiers cGMP and cADPR.

Cross-talk among NO, salicylic acid and jasmonic acid

Several lines of evidence point to an inter-relationship between NO and salicylic acid (SA) in plant defence (Figure 1). Treatment of tobacco and *A. thaliana* leaves with NO induces a substantial increase in endogenous SA [4,23]. In tobacco, this increase is required for PR-1 expression and probably involves NO-dependent induction of the *PAL* gene [4]. In addition, NOS inhibitors and a NO scavenger attenuate SA-induced systemic acquired resistance (SAR) [24]. Although these results suggest that NO is involved in both SA biosynthesis and action, other studies have indicated that NO function requires SA. In transgenic tobacco, the ability of NO donors to reduce the size of TMV-induced lesions was abolished by the expression of the bacterial gene *nahG*, which encodes the SA-degrading enzyme salicylate hydroxylase [24].

Recent evidence suggests that NO also plays a role in the wounding/jasmonic acid (JA) signalling pathway (Figure 1). In tomato, NO donors inhibited both wounding-induced H\(_2\)O\(_2\) synthesis and wounding- or JA-induced expression of defence genes [25]. This inhibition was independent of SA, which has been shown to antagonise JA synthesis and/or activity. Thus, NO may interact directly with the wounding/JA pathway at a point downstream of JA synthesis and upstream of H\(_2\)O\(_2\) generation. Consistent with this possibility, NO donors delayed and/or reduced wounding-induced generation of H\(_2\)O\(_2\) and expression of the JA-inducible ipomoelin gene in sweet potato [26]. Additional evidence that NO cross-talks with the wounding/JA pathway comes from the demonstration that wounding- and/or JA treatment induces NO production in sweet potato and *A. thaliana* epidermal cells [23,26], and that exogenous NO induces all of the genes that are required for JA biosynthesis (Table 2). The

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**Figure 1**

NO functions in ABA, defence and wounding signalling. In guard cells, both NR and AtNOS1 have been proposed to catalyse ABA-induced NO synthesis. NO activates intracellular Ca\(^{2+}\)-permeable channels through a cGMP/cADPR-dependent pathway. The resulting increase in free cytosolic Ca\(^{2+}\) concentration leads to the inactivation of inward-rectifying K\(^{+}\) channels (I\(_{K}\)), which suppresses K\(^{+}\) influx, and to the activation of Cl\(^{-}\) channels (E\(_{Cl}\)), which allows anion efflux. The resulting large efflux of anions would result in long-term depolarisation of guard cells, which in turn activates outward-rectifying K\(^{+}\) channel currents, leading to a reduction of turgor pressure and therefore stomatal closure. In response to pathogens or other elicitors of plant defences, NO is produced by iNOS, a variant of the P protein of the glycine decarboxylase complex. NO utilises at least four pathways, whose interconnections are not well understood, (a) to elevate free cytosolic Ca\(^{2+}\) through a signalling cascade similar to that reported in guard cells, (b) to induce the HR/cell death in cooperation with H\(_2\)O\(_2\), (c) to induce SA production, which in turn enhances NO levels and facilitates local resistance and the development of systemic acquired resistance (SAR), and (d) to induce the expression of defence genes through SA- and Ca\(^{2+}\)-dependent pathway(s), and perhaps also through SA- and Ca\(^{2+}\)-independent pathway(s).

NO also has been associated with wounding responses. Both wounding and JA induce NO synthesis through an enzyme that has not yet been characterised. Conversely, in *A. thaliana*, NO activates genes that encode biosynthetic enzymes involved in JA production. NO-induced synthesis of JA was only observed in SA-deficient plants, however, suggesting the existence of a self-amplifying JA–NO loop that is negatively regulated by SA. SA also may repress NO-induced expression of JA-responsive genes. In species other than *A. thaliana*, including tomato and potato, NO downregulates JA- and wounding-responsive genes.
relationship between these pathways remains unclear, however, because NO treatment did not increase JA levels and the wounding-induced expression of JA-forming enzymes was independent of NO [23]. Interestingly, NO treatment of SA-deficient NahG plants resulted in the activation of JA-responsive genes and JA production, suggesting that SA negatively regulates NO-mediated JA synthesis in wildtype plants.

**NO and cell death**

Evidence that NO plays a role in plant cell death is accumulating. Treating suspension-cultured Citrus sinensis cells with NO donors induces cell death. This cell death shares similarities with programmed cell death (PCD) in animal cells, including chromatin condensation and loss of mitochondrial membrane electrical (PCD) in animal cells, including chromatin condensation and loss of mitochondrial membrane potential [27]. Moreover, mammalian NOS inhibitors reduced the PCD that occurred during the hypersensitive response (HR) in A. thaliana plants that were challenged by avirulent P. syringae [3]. Similarly, tobacco plants and alfalfa root cultures that over-produced haemoglobin, which can act as an NO scavenger, exhibited reduced cell death after inoculation with avirulent pathogens [28] or under hypoxic conditions [29], respectively. In soybean cells, increased levels of NO are not sufficient to trigger cell death in the absence of other reactive oxygen species (ROS) [30]. In animal cells, PCD is mainly mediated by peroxynitrite (ONOO⁻) that is formed from NO and superoxide (O₂⁻•). In contrast, HR-associated cell death in soybean cells appears to be mediated by the relative level of NO and H₂O₂ that is formed by dismutation of O₂⁻• [30]. Consistent with this conclusion, only the simultaneous increase of NO and H₂O₂ in tobacco cells induced cell death that had typical cytological and biochemical features of PCD [31].

These studies suggest that NO regulates HR cell death, but NO synthesis may not be a prerequisite for initiating the PCD signalling pathway. NO production in P. syringae-inoculated A. thaliana did not precede the HR, but rather occurred concurrently with HR [32]. Because NO was first detected in the extracellular spaces, and then in the cytoplasm of nearby cells that died soon afterwards, it was proposed that NO facilitates the cell-to-cell spread of the HR.

NO also has been shown to display anti-apoptotic properties. NO protects barley aleurone layers against gibberellin-induced cell death [33] and wheat seedlings from drought [22]. The mechanisms through which NO exerts its plethora of effects are not well understood, but several studies indicate that NO protects cells from ROS-mediated cellular damage and cytotoxicity by increasing the levels of cyto-protective proteins, including catalase, superoxide dismutase, glutathione S-transferase and alternative oxidase (Table 2; [33,34*,35*]). Furthermore, by abrogating O₂⁻•-mediated cytotoxic effects through the conversion of O₂⁻• into ONOO⁻, NO might provide protection against oxidative stress [30].

To summarise, NO appears to be a bifunctional modulator of plant cell death that is capable of either stimulating or inhibiting this process, as has been previously documented in animal systems. The data discussed here strongly suggest that the cross-communication of

<table>
<thead>
<tr>
<th>Classes of NO-regulated genes in plants.</th>
<th>Effect on expression</th>
<th>NO source</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzymes involved in JA synthesis</td>
<td>+</td>
<td>Gaseous NO</td>
<td>[23]</td>
</tr>
<tr>
<td>JA-responsive genes</td>
<td>-</td>
<td>NO donors, gaseous</td>
<td>[25,26]</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>NO</td>
<td>[23]</td>
</tr>
<tr>
<td>Enzymes involved in ethylene synthesis</td>
<td>+</td>
<td>NO donor</td>
<td>[34*]</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Cryptogein</td>
<td>[11]</td>
</tr>
<tr>
<td>Proteins involved in ethylene signalling</td>
<td>+</td>
<td>NO donor</td>
<td>[35*]</td>
</tr>
<tr>
<td>PR genes</td>
<td>+</td>
<td>NO donors</td>
<td>[4,34*,35*]</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>TMV</td>
<td>[19]</td>
</tr>
<tr>
<td>Enzymes of the phenylpropanoid pathway</td>
<td>+</td>
<td>NO donors</td>
<td>[4,35*]</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>P. syringae</td>
<td>[3]</td>
</tr>
<tr>
<td>Anti-oxidant and other protective proteins</td>
<td>+</td>
<td>NO donors</td>
<td>[34*,35*,40]</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Cryptogein</td>
<td>[11]</td>
</tr>
<tr>
<td>Proteins involved in photosynthesis</td>
<td>+</td>
<td>NO donor</td>
<td>[35*]</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>NO donor</td>
<td>[35*]</td>
</tr>
<tr>
<td>Proteins involved in cellular trafficking</td>
<td>+</td>
<td>NO donor</td>
<td>[35*]</td>
</tr>
<tr>
<td>Putative cell death proteins</td>
<td>+</td>
<td>NO donor</td>
<td>[35*]</td>
</tr>
<tr>
<td>Proteins for basic metabolism</td>
<td>+ or -</td>
<td>NO donors</td>
<td>[34*,35*]</td>
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<tr>
<td>Auxin-responsive proteins</td>
<td>+</td>
<td>NO donor</td>
<td>[34*]</td>
</tr>
<tr>
<td>Ferritin</td>
<td>+</td>
<td>NO donor</td>
<td>[41]</td>
</tr>
<tr>
<td>Genes of unknown function</td>
<td>+</td>
<td>NO donors</td>
<td>[34*,35*]</td>
</tr>
</tbody>
</table>

*+*, upregulation by NO; −, downregulation by NO. *Only in SA-deficient NahG transgenic Arabidopsis.*
NO with other pro-oxidants or anti-oxidants critically influences the fate of cells that are challenged by cell-death mediators.

Conclusions and future perspectives
Studies on the function of NO in hormonal and defence signalling, as well as in the modulation of cell death, have revealed important new biochemical and molecular information about this essential physiological mediator (Figure 1). The observation that NO regulates the expression of many genes (Table 2) suggests that it affects numerous physiological processes in plants. The recent discovery of two plant NOS that have little sequence similarity to each other or to their mammalian counterparts, together with the demonstration that NR is an important source of NO in some physiological processes, argues that although both plants and mammals use multiple enzymes for NO production, the chemistry of NO synthesis in plants differs from that in mammals. The evidence presented to date suggests that crucial players in animal NO signalling also operate in plants. These include two major direct targets of NO in animals, GC and aconitase [36], and the second messengers cGMP, cADPR and Ca$^{2+}$. Given these similarities, it is likely that plant NOS and the downstream NO effectors, like those in mammals, are part of macromolecular complexes in which NO functions within highly localised environments. Such spatial contiguity may determine the efficiency and specificity of signal propagation. The major challenges ahead are to determine which of the three (or more) NO-producing enzymes in plants participates in a particular physiological process, and how the correct specific response is evoked despite shared use of the NO signal and, in some cases, its downstream second messengers. We anticipate that specificity may involve the differential subcellular localisation of iNOS, AtNOS1 and NR (and perhaps also the NO targets), channelling of the signal (perhaps via macromolecular complexes), and differences in the amplitude and/or duration of the NO signal.

Another crucial area that has received little attention is the identification and characterisation of the direct targets of NO. These are likely to include not only GC and aconitase but also intracellular Ca$^{2+}$-permeable channels and proteins that mediate pro-/anti-apoptotic processes. The identification of new NO targets will not be easy; however, this strategy has proven fruitful for addressing questions concerning the effects of NO in animals [1]. Given the dramatic increase in our appreciation of the role of NO in plants over the past half decade, and the myriad effects of NO that have been documented in animals since its discovery as the endothelium-derived relaxation factor 17 years ago [37,38], we clearly have just begun to tap an immense well of knowledge that should provide a deeper understanding of the biology of plants.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

This contribution represents a major breakthrough by identifying iNOS as the first known pathogen-inducible NOS enzyme in plants. The activity of this protein is dependent on NADPH, calmodulin, flavin, heme and tetrahydrobiopterin. The A. thaliana ortholog of iNOS shares 89% identity with the A. thaliana P protein of the GDC. iNOS resembles mammalian inducible NOS in that it uses the same co-factors, has comparable kinetic properties, and is induced by pathogens. However, most of the critical co-factor-binding sites found in mammalian NOS are not readily evident.
in iNOS, suggesting that the plant enzyme uses distinct chemistry for NO synthesis. Because of their unexpected nature, both iNOS and AtNOS1 [55] define new classes of NOS.


This work provides the first direct in-vivo evidence that the plant iNOS [10] contributes to host defence against pathogens. Virus-induced gene silencing using LeprP, a putative ortholog of A. thaliana iNOS [10], led to enhanced susceptibility to P. syringae pv. tomato (P.s.t.) in both resistant and susceptible tomato. Increased susceptibility correlated with a dramatic reduction in LeprP transcripts, a complete suppression of the induction of NOS activity by P.s.t., and an 80% reduction in GDC activity. To address whether the enhanced susceptibility to P.s.t. was caused by the loss of iNOS, of GDC or both activities, the authors analysed symptom development and P.s.t. growth in susceptible plants in which both P.s.t. and GDC activity was entirely macromolecular. The inhibition of both enzymes resulted in enhanced disease symptoms and P.s.t. growth, whereas susceptibility was unchanged in plants in which only GDC activity was inhibited.


The authors make an outstanding contribution to the field by providing genetic, pharmacological and physiological evidence of the role of NO as a source of NO in ABA signalling in A. thaliana guard cells. The generation of NO after the treatment of guard cells with either ABA or nitrate, a substrate for NR, correlated with stomatal closure. Both ABA and nitrate failed to induce stomatal closure in epidermal peels of the NR double mutant nia1 nia2, arguing that NR is required to generate NO that subsequently mediates ABA-induced stomatal closure.


This paper provides the first evidence that NO is produced non-enzymatically in the apoplast. The authors show that NO is rapidly synthesised by the chemical reduction of nitrate in the apoplast of barley aleurole layers. Nitrite-dependent, non-enzymatic NO synthesis required an acidic pH and was observed in response to gibberellic acid and ABA, two hormones that rapidly acidified the apoplastic medium. Furthermore, the chemical production of NO was accelerated by reducing agents, including phenolic compounds.


The authors unravel the contribution of NO as a signalling molecule in the ABA transduction pathway that leads to stomatal closure. They report that NO selectively activates intracellular Ca2+ -permeable channels in Vicia faba guard cells through a cGMP/cADPR-dependent signalling pathway. The resulting elevation of cytosolic Ca2+ is required for the inactivation of inward-rectifying K+ channels and the activation of outward-rectifying Cl- channels at the plasma membrane, two key events in ABA-induced stomatal closure.


Together with [35], this paper describes a highlight of recent work on NO signalling in plants. The authors studied the transcriptional changes mediated by artificially generated NO in A. thaliana suspension cells by using a cDNA microarray that included about 200 defence-related genes and 50 genes that are associated with primary metabolism. About 5% of the transcripts showed transient changes in expression. Amongst the NO-sensitive genes, NO strongly induces a gene that encodes alternative oxidase, a protein that might act to counteract NO-induced inhibition of cytochrome c-dependent respiration.

cDNA-amplification fragment length polymorphism (AFLP) analysis identified transcripts whose expression was rapidly altered following infiltration of A. thaliana leaves with a NO donor. Of approximately 2500 cDNAs analysed, 120 differentially regulated cDNAs were detected. In addition to the expected activation of genes that are related to defence responses and oxidative stress, a large number of genes that are associated with metabolism and signalling were also expressed in response to NO treatment.


