Lehrstuhl für Botanik
der Technischen Universität München

Gene Induction by Nitric Oxide (NO) in *Arabidopsis thaliana*

Xi Huang

Vollständiger Abdruck der von
der Fakultät Wissenschaftszentrum Weihenstephan
für Ernährung, Landnutzung und Umwelt der Technischen Universität München
zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften
(Dr. rer. nat.) genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. E. F. Elstner

Prüfer der Dissertation:
1. Univ.-Prof. Dr. E. Grill
2. Priv.-Doz. Dr. J. Durner

Die Dissertation wurde am 25. 09. 2003 bei der Technischen Universität München
eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan
für Ernährung, Landnutzung und Umwelt am 13. 11. 2003 angenommen.

Finished in

Institute of Biochemical Plant Pathology

GSF - National Research Center for Environment and Health

Under Supervision by PD Dr. Jörg Durner

Index

Abbreviations

aa	Aminoallyl
AOS	Allene oxide synthase
AOX	Alternative oxidase
avr	Avirulence gene
BH4	Tetrahydrobiopterin
BIOP	Institute of Biochemical Plant Pathology
bp	Base pairs
BSA	Bovine serum albumin
°C	Degree Celsius
CaM	Calmodulin
cDNA	Complementary DNA
COX	Cytochrome c oxidase
cPTIO	Carboxy-2-phenyl-4,4,5,5-tetramethylimidazolinone-3-oxide-1-oxyl
Cy	Cyanine dye
d	distilled
DAF-2 DA	4, 5-Diaminofluorescein diacetate solution
DEPC	Diethyl cyanophosphonate
DETC	Sodium diethyldithiocarbamate hydrate
DMSO	Dimethylsulfoxid
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotid-5'-triphosphate
EDRF	Endothelium derived relaxing factor
EDTA	Ethylendiaminetetraacetic acid
e. g.	For example
EPR	Electron paramagnetic resonance
EST	Expressed sequence tag
et al.	and others
etr1	Ethylene resistant mutant
GDC	Glycine decarboxylase complex
GST	Glutathione S-transferase
h	Hour

HR	Hypersensitive response
IRP	Iron regulatory protein
JA	Jasmonic acid
jar1	Jasmonate resistant mutant
jin1	Jasmonate insensitive mutant
JIP	Jasmonate inducible protein
kD	Thousand dalton
l	Liter
LAP	Latency-associated peptide
LOX	Lipoxygenase
M	Molar
mg	Milligram
MJ	Methyl jasmonate
ml	Milliliter
mM	Millimolar
MOPS	4-Morpholinepropanesulfonic acid; 3-Morpholinopropanesulfonic acid
mRNA	Messenger RNA
µ	Micron
µl	Microliter
µM	Micromolar
NahG	Transgenic plants containing bacterial salicylate hydroxylase gene
NaR	Nitrate reductase
NASC	Nottingham *Aiabidopsis* Stock Centre
NF-κB	Nuclear factor kappa B
nM	Nanomolar
NO	Nitric oxide
NOS	Nitric oxide synthase
NOR3	(+/-)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide
NPR1	Nonexpresser of PR genes
opr	12-oxo-phytodienoic acid reductase
PAD4	Phytoalexin deficient mutant
PAL	Phenylalanine ammonia lyase
PBS	Phosphate-buffered saline
PCD	Programmed cell death

PCR	Polymerase chain reaction
PD	Privatdozent
PDF1.2	Defensin 1.2
pH	Negative decadic logarithm of the molar concentration of hydrogen ions
ppm	parts per mille
PR	Pathogen-related protein
PVP	Polyvinylpyrrelidon
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Rotations per minute
SA	Salicylic acid
sai1	Salicylic acid insensitive mutant
SAR	Systemic acquired resistance
SD	Standard deviation
SDS	Sodium dodecyl sulfate
sGC	Soluble isoform of guanylyl cyclase
SHAM	salicylhydroxamic acid
SNP	Sodium nitroprusside
ssDNA	Salmon sperm DNA
TAE	Tris-acetate-EDTA
TBE	Tris-boric acid-EDTA
TE	Tris-EDTA
TGF	Transforming growth factor
TM	Trademark
UV	Ultra violet
VSP	Vegetative storage protein
v/v	Volume per volume
wt	Wild type
w/v	Weight per volume
www	World wild web

I. Introduction

During the past two decades, one of the most vigorously researched and intriguing entities of biological chemistry is nitric oxide (NO, often also abbreviated as ˙NO to indicate that it is a free radical with a unpaired electron), a unique type of biological messenger in animal cells. Previously NO's notoriety stemmed primarily from the recognition of its presence in the environmentally polluting NO_x complex (NO_2 and NO), the component of which, via the Chapman pathway converts stratospheric and tropospheric O_3 to O_2 (Howard, 1980; Sandermann, 2001). In 1987, vascular 'endothelium derived relaxing Factor' (EDRF) was identified as NO by team headed by Salvador Moncada in UK (Palmer et al., 1987). This finding is regarded as the groundbreaking one serving as a cornerstone for subsequent research on NO. From then on, NO has attracted a great deal of attention. Nitric oxide became the sources of intensive and exciting research in animal. In 1992, NO was recognized by *Science* magazine as the 'Molecule of the year'. Further investigations led to the finding that NO is a multifunctional effectors in numerous mammalian physiological processes, including the relaxation of smooth muscle, inhibition of platelet aggregation, neural communication and immune regulation (Schmidt and Walter, 1994). Many insights into the understanding of NO functions came from the identification of nitric oxide synthase (NOS), the enzyme responsible for NO production (Nathan and Xie, 1994). Moreover, studies of NO chemistry have contributed to an understanding of NO signaling mechanisms that are achieved through its interaction with targets via a rich redox and additive chemistry (Stamler, 1994; Stamler et al., 1992). In 1998, three NO research pioneers (Robert F. Furchgott, Louis J. Ignarro and Ferid Murad) won the Nobel Prize for "Physiology or Medicine" for their discoveries concerning "the nitric oxide as a signaling molecule in the cardiovascular system".

The use of NO is not confined to the animal kingdom. The ability of plants to accumulate and metabolize atmospheric NO has been known for some time (Nishimura et al., 1986). Moreover, measurement of gaseous emissions from plants have shown that NO can be synthesized in plants, through both non-enzymatic and enzymatic reactions (Leshem and Haramaty, 1996; Yamasaki, 2000). Interestingly, studies conducted in the past several years show that NO is at the heart of several physiological functions ranging from plant development to defense responses (Beligni and Lamattina, 2000; Durner and Klessig, 1999). Remarkably, these analyses suggest that many aspects of NO signaling are shared by plants

and animals. Interestingly, a pathogen-inducible nitric oxide synthase (iNOS) in plant was identified and cloned very recently (Chandok et al., 2003). Despite the lack of sequence homology, the biochemical properties of this plant enzyme, including cofactor requirements and inhibitor sensitivity, as well as its kinetic properties, appear to be very similar to those of its animal counterparts. Moreover, plant iNOS, like that of animals, is specifically induced during the resistance response to pathogen infection.

NO, first identified as EDRF, is now recognized as one of the most versatile mediators in bacteria, protozoa, animal and plant. The following sections will endeavor to highlight the current progress in NO research and clarify the goal of this work.

1. Chemical basis for NO-mediated signaling effects

NO has been known as a ubiquitous inter- and intracellular messenger. NO controls a variety of complex biological processes, including blood pressure homeostasis, platelet aggregation, and transmission of signals by the nervous system. NO is also important for immune system function, playing key roles in the activation of macrophages and cellular defenses against microbial pathogens (Aslan and Freeman, 2002). How can NO function as a signaling molecule? NO chemistry implicates an interplay between the three redox-related species: nitric oxide radical (NO), nitrosonium cation (NO^+) and nitroxyl anion (NO^-). In biological systems, NO reacts rapidly with molecular oxygen (O_2), superoxide anion (O_2^-) and transition metals. The reaction of NO with O_2 results in the generation of NO_x compounds (including NO_2., N_2O_3 and N_2O_4), which can either react with cellular amines and thiols, or simply hydrolyze to form the end metabolites nitrite (NO_2^-) and nitrate (NO_3^-) (Wendehenne et al., 2001). NO can entertain several complex chemical reactions, some of which have been shown to be associated with certain biological effects of NO.

1.1. Peroxynitrite formation

The reaction of NO with O_2^- yields peroxynitrite ($ONOO^-$). Peroxynitrite is a strong oxidant that mediates cellular injury. At physiological pH, $ONOO^-$ equilibrates rapidly with pernitrous acid (ONOOH), which, depending on its conformation, rapidly decomposes to NO_3^- or to the highly reactive hydroxyl radical HO (Wendehenne et al., 2001). $ONOO^-$ can also oxidize thiol residues to sulfenic and sulfonic acids and nitrate peptides and proteins at the phenyl side chain of tyrosine residues. Tyrosine nitration is a widely used marker of peroxynitrite

produced from the reaction of nitric oxide with superoxide (Reiter et al., 2000). However, the molecular targets of peroxynitrite and the functional consequences of peroxynitrite-mediated tyrosine nitration *in vivo* (including possible effects on cellular signaling cascades) have only begun to be defined.

1.2. Formation of S-nitrosylation

NO is extremely susceptible to both oxidation and reduction. One electron oxidation of NO leads to NO^+. This oxidation can be supported by Fe(III)-containing metalloproteins. NO^+ mediates electrophilic attack on reactive sulfur, oxygen, nitrogen and aromatic carbon centers, with thiols being the most reactive groups. This chemical process is referred to as nitrosylation. Nitrosylation of sulfhydryl (*S*-nitrosylation) centers of many enzymes or proteins have been described and the resulting chemical modification affects activity in many cases. Considering that, within complex proteins such as enzymes and transcription factors, cysteine residues and the formation or disruption of disulfide bridges are frequently crucial for tertiary structure and function, it is evident that S-nitrosylation is a valid mechanism for the signaling effects of NO (Bogdan, 2001b; Stamler and Hausladen, 1998; Wendehenne et al., 2001).

1.3. Reaction with transition metals

NO is capable of donating electrons and therefore reacts with transition metals such as iron, copper and zinc, which leads to the formation of metal-nitrosyl complexes. Transition metals are not only components of prosthetic groups of enzymes and other proteins (e.g. iron of the heme moiety of soluble isoform of guanylyl cyclase [sGC], hemoglobin or myoglobin) but also coordinate sulfide clusters in enzymes (e.g. [Fe-S] proteins of the respiratory chain), transcription factors (e.g. zinc-finger proteins) and mRNA-binding proteins (e.g. iron-regulatory protein 1) (Durner et al., 1999; Gow et al., 1999; Shinyashiki et al., 2000; Zhao et al., 1999). Again, NO was found to either activate or inhibit the function of the respective proteins (e.g. in the case of the zinc-finger domains).

2. NO signaling in mammalian systems

Progress of NO research in mammalian system is earlier and more prosperous than in plant system. Of course research in medicine occupies more resources than in plant research. The advance of NO research in animal has directly inspired and promoted the research in plants. For example, using animal NOS, NOS inhibitors to study plant NO signaling has reached exiting success (Delledonne et al., 1998; Durner et al., 1998). So it is very necessary to overview the present state of the NO research in animal, before I try to draw up the work scheme about the NO signaling in plants.

2.1. Nitric oxide biosynthesis in mammals

To fully understand the regulation of NO, the first urgent task is to reveal the origin of NO. In animals, NO is generated by nitric oxide synthases (NOS), a group of evolutionarily conserved cytosolic or membranebound isoenzymes that convert the amino acid L-arginine to citrulline and NO (Nappi et al., 2000; Stuehr, 1999). Three NOS isoforms have been identified (Nathan and Xie, 1994), named on the basis of the tissue source from which they were originally extracted: neuronal NOS (nNOS or NOS1), inducible NOS in macrophages (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). Recently, a new isoform of NOS was found in mitochondria isolated from rat liver (Tatoyan and Giulivi, 1998). This isoform, named mtNOS, is similar to iNOS. All NOS isoforms are homodimeric enzymes that require the same cosubstrates (molecular oxygen, NADPH) and cofactors (FMN, FAD, tetrahydrobiopterin, heme, Ca^{2+}/calmodulin, and, possibly, also Zn^{2+} ions) and show ~50-60% identity in their amino acid sequences (Stuehr, 1999).

The structure and function of NOS is schematic represented in **Figure 1**. Each NOS is a bi-domain enzyme consisting of an N-terminal oxygenase and a C-terminal reductase (Mayer and Hemmens, 1997). The oxygenase domain contains a cytochrome P-450 type heme center and a binding site for the cofactor tetrahydrobiopterin (BH4). The reductase domain contains NADPH, FAD and FMN binding sites and exhibits significant homology with NADPH cytochrome P-450 reductase. Both domains are connected by a calmodulin (CaM) binding site in the middle of the enzyme. In addition, each NOS has a different N-terminal extension determining the intracellular localization of the enzyme (Mayer and Hemmens, 1997).

The nNOS and the eNOS are referred to as constitutive NOSs, whereas iNOS gene expression is induced in macrophages and many other cell types in response to inflammatory agents and cytokines. The high amounts of NO produced by iNOS exert cytotoxic and antimicrobial effects on the immune system (Mayer and Hemmens, 1997; Nathan and Xie, 1994; Wendehenne et al., 2001).

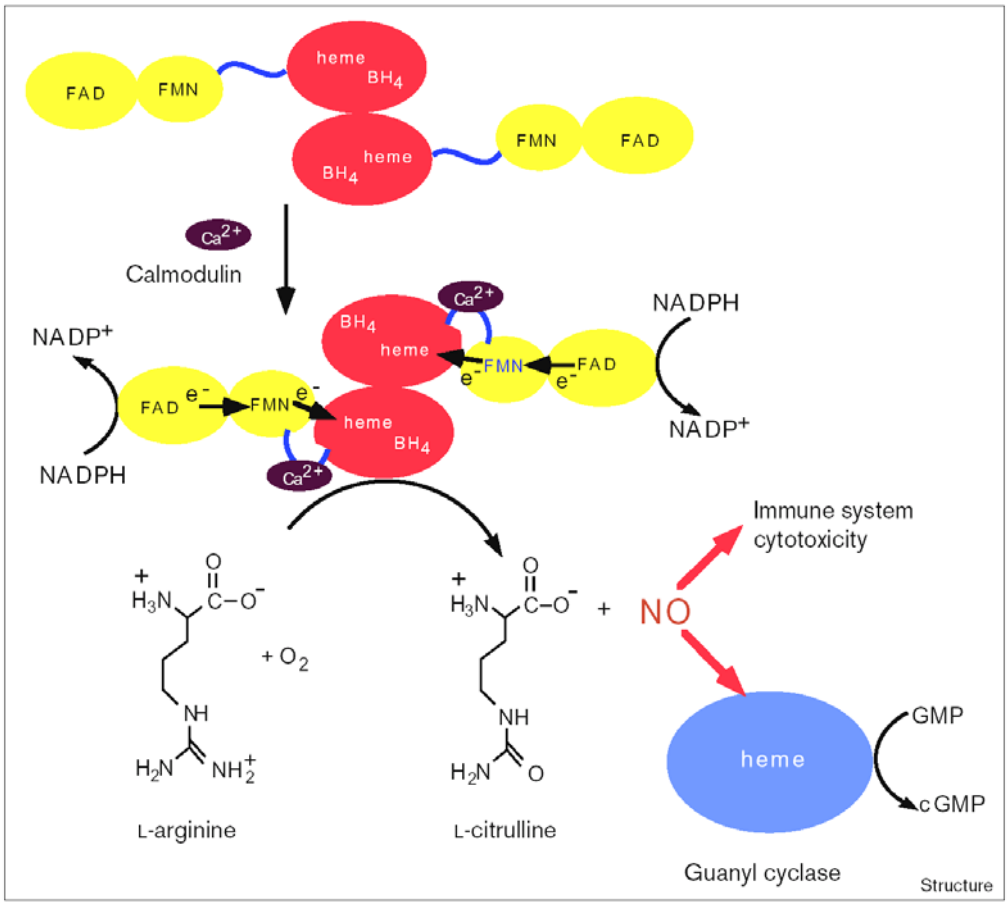


Figure 1: Schematic representation of NOS structure and how NOS functions (Figure adapted from Poulos et al., 1998).

2.2. Regulation of gene expression by nitric oxide

The biological activities of NO are numerous and complex. But how does NO play its role is yet to be fully elucidated. A classical example for the signaling effects of NO is the relaxation of vascular smooth muscle cells when they are exposed to NO produced by adjacent endothelial cells. This effect, which was the first function of NO to be discovered, is due to the activation of the soluble isoform of guanylyl cyclase (sGC), the formation of cGMP and the subsequent activation of cGMP-dependent ion channels and kinases (Bellamy et al., 2000;

Zhao et al., 1999). However, sGC is not the only 'receptor' for NO. During the past years, multiple other target molecules have been identified, many of which are part of intracellular signal-transduction cascades leading to modulation of certain genes. To date, there is no evidence for the existence of DNA elements within the promoters region of eukaryotic genes that respond directly to NO. Most, if not all, of these effects of NO are indirect. They are based on the modulation of transcription factors, of the translation or stability of mRNA, or of the processing of the primary (functionally inactive) gene product (Bogdan, 2001b). Recent examples of NO regulated gene expression in mammalian system will be summarized in the following sections.

2.2.1. NO and DNA-methylation

Methylation status of control regions in the genome plays a critical role in the regulation of gene expression. In eukaryotic cells, cytosine 5'-methylation of CpG-dinucleotides favors a repressive chromatin structure that impedes the promoter binding of transcriptional activators and causes gene silencing (Bird, 1992). Fragile X mental retardation gene (*FMR1*) and hypoxanthine phosphoribosyltransferase (*HPRT*) have a CpG island in their promoter region. The repression of these genes by interleukin (IL)-1ß could be fully prevented by iNOS inhibitors. NO donors also caused *FMR1* and *HPRT* gene silencing by methylation of CpG island. This effect could be reverted by demethylating agents, which, in turn, produced the recovery of gene expression (Hmadcha et al., 1999).

2.2.2. NO and transcription factors

NO regulates gene expression on transcriptional level mainly through modulating the transcriptional factors. In the bacterial system, studies on SoxR and OxyR, two transcriptional activators of genes encoding antioxidants, have yielded rather uniform results showing that reactive nitrogen intermediates (as well as reactive oxygen species) can induce and activate these two resistance mechanisms (e.g. in *Escherichia coli* and *Salmonella typhimurium*) (Ding and Demple, 2000; Hausladen et al., 1996).

In eukaryotic system, the results seem to be disputed. The paradigmatic NO mediated eukaryotic transcription factor is nuclear factor kappa B (NF-κB). NF-κB is a dimeric transcription factor that is sequestered in the cytoplasm by tightly bound inhibitory proteins called IκBs (Verma et al., 1995). It is best known for its stimulatory functions in the immune

system, notably in B-cells and macrophages. Activation of these cells leads to an IκB kinase (IKK)-mediated phosphorylation, ubiquitination and proteosomal degradation of IκB, which then allows the translocation of free NF-κB into the nucleus (Stancovski and Baltimore, 1997). There are considerable body of evidence that NO (endogenous NO or NO donors) can modulate the process of NF-κB activation. But the obtained results are far less clear and even contradictory, namely, NO can activate or inhibit NF-κB (Deora et al., 1998; Matthews et al., 1996; Spiecker et al., 1997; Umansky et al., 1998).

2.2.3. NO effects mRNA stability and translation

NO-dependent posttranstriptional mechanisms of gene regulation have been well documented. A best known example is iron homeostasis control. The cellular iron homeostasis is maintained by iron-regulatory protein (IRP) that was determined to be the cytosolic isoform of aconitase (Kaptain et al., 1991). IRP binds to iron-responsive elements (IREs) that are located in the 5'-untranslated regions of the mRNA of ferritin, and thereby regulates their translation. NO converts the cytosolic aconitase into an IRP by promoting the loss of the iron-sulfur cluster, which otherwise prevents IRE binding. Consequently, the activities of aconitase and IRP are mutually exclusive and regulated by in part by NO (Hentze and Kuhn, 1996).

2.2.4. Regulation of posttranslational events by NO

Tumor cells often produce abundant transforming growth factor ß1 (TGF-ß1), which suppresses the inducible isoform of NO synthase. TGF-ß1 is secreted in a latent form, which consists of TGF-ß1 noncovalently associated with latency-associated peptide (LAP). TGF becomes active upon disassembly of this complex, which can be elicited by acid pH, enzymes (e.g. plasmin) or oxidants including hemin and nitroxyl anion (NO^-). LAP, however, is able to reassociate with and neutralize the bioactivity of TGF. Recently, Vodovotz *et al.* have demonstrated that the latter process is controlled by NO radicals through their inactivation of LAP by S-nitrosylation. Active TGF or the latent TGF- complex, by contrast, were not affected by NO (Vodovotz et al., 1999).

2.3. NO signaling and animal immune response

When nitric oxide (NO) formally entered the immunology scene, between 1985 and 1990, its role in the immune system was simply defined: NO is a product of macrophages activated by cytokines, microbial compounds or both, is derived from the amino acid L-arginine by the enzymatic activity of inducible nitric oxide synthase (iNOS or NOS2) and functions as a tumoricidal and antimicrobial molecule *in vitro* and *in vivo* (Nathan, 1992). Although this basic definition is still accepted, during the past decade it has been recognized that NO plays many more roles in the immune system as well as in other organ systems. There are a number causes for this. First, not only macrophages, but also a large number of immune-system cell (dendritic cells, natural killer (NK) cells, mast cells and phagocytic cells including monocytes, macrophages, microglia, Kupffer cells, eosinophils, and neutrophils) produce and response to NO. Second, contrary to previous views, all known isoform of NO synthase (nNOS, iNOS and eNOS) operate in the immune system. Third, the activity of NO is not restricted to the site of its production. No is highly diffusible and can combine with other molecules to function as long distance signaling (e.g. GSH - GSNO). Finally, in contrast to cytokines, the interaction of NO is not restricted to a single receptor (Bogdan, 2001a).

NO has been recognized as one of the most versatile players in the immune system (**Table 1**). It is involved in anti-microbial activity; anti-tumor activity; tissue-damaging effects; anti-inflammatory-immunosuppressive effects; modulation of the production and function of cytokines, chemokines, and growth factors and T helper cell deviation. But most studies of NO have involved exogenous NO sources (and arbitrarily chosen NO concentration) and NOS inhibitors with possible side effects, and have been carried out a wide range of cell types and cell-free systems. It has therefore been impossible to estimate the true extent to which NO exerts positive or negative signaling effects. Because of variety of reaction partners of NO, its widespread production and the fact that its activity is strongly influenced by its concentration, NO continues to surprise and perplex NO researchers (Bogdan, 2001a).

Table 1. Overview of immune-system NO function (Table adapted from Bogdan, 2001a).

Category	Producers of NO (examples)	Phenotypic effect of NO	Examples of underlying molecular mechanisms
		Effector functions	
Antimicrobial activity	Macrophages, microglia, neutrophils, eosinophils, fibroblasts, endothelial cells, epithelial cells, astroglia	Killing or reduced replication of infectious agents (viruses, bacteria, protozoa, fungi, helminths)	•Direct effect of NO on the pathogen •Indirect effects of the NOS pathway (*e.g.*, reaction of NO with other effector molecules, arginine depletion; see text)
Anti-tumor activity	Macrophages, eosinophils	Killing or growth inhibition of tumor cells	•Inhibition of enzymes essential for tumor growth (*e.g.*, enzymes of the respiratory chain, *cis*-aconitase, ribonucleotide reductase arginase, ornithine decarboxylase) •Growth inhibition *via* iNOS-dependent depletion of arginine •Cell-cycle arrest (downregulation of cyclin D1) •Induction of apoptosis (by activation of caspases and accumulation of p53) •Sensitization of tumor cells for TNF-induced cytotoxicity
Tissue-damaging effect (immunopathology)	Macrophages, microglia, astroglia, keratinocytes, mesangial cells	Necrosis or fibrosis of the parenchyma	•Apoptosis of parenchymal cells •Degradation of extracellular matrix •Deposition of matrix, proliferation of mesenchymal cells •Influx of inflammatory cells via chemokine regulation
		Immunoregulatory functions	
Anti-inflammatory–immunosuppressive effect	Macrophages ('suppressor phenotype')	Inhibition of: •T cell proliferation •B cell proliferation •Antibody production by $CD5^+$ B cells •Autoreactive T and B cell diversification Inhibition of leukocyte recruitment (adhesion, extravasation, chemotaxis)	•Apoptosis of T cells or APCs •Downregulation of MHC class II, costimulatory molecules or cytokines •Disruption of signaling cascades and transcription factors •Inhibition of DNA synthesis •Downregulation of adhesion molecules or chemokines
Modulation of the production and function of cytokines, chemokines, and growth factors (pro- or anti-inflammatory effects)	Macrophages T cells endothelial cells fibroblasts	Up- and downregulation, *e.g.*, of: •IL-1, IL-6, IL-8, IL-10, IL-12, IL-18, IFN-γ, TNF •TGF-β, G-CSF, M-CSF, VEGF, •MIP-1α, MIP-2, MCP-1	Modulation of •Signaling cascades (*e.g.* G-proteins, Jak, MAP kinases, caspases, protein phosphatases) •Transcription factors (*e.g.* NF-κB, Sp1, AP-1) •Proteins regulating mRNA stability or mRNA translation •Latent cytokine precursor complexes •Enzymes that process cytokine precursors
T helper cell deviation	*e.g.*, macrophages	•Induction and differentiation of T_H1 cells •Suppression of T_H1 (and T_H2) cell responses •Suppression of tolerogenic T cell responses	1. Possible stimulation of IL-12-mediated signaling 2. Suppression of IL-12 production

3. NO signaling in plants

When NO entered plant biologist's field of vision, NO was regarded as a polluting gas. Combustion is a classic source of NO_x (NO and NO_2). The atmospheric NO_x likely enters plants through stomata, or maybe by penetrating the damaged cuticle. Fumigating the plants to atmospheric NO_x led to a series of biochemical and physiological changing in plants, e. g. nitrate reductase and nitrite reductase activation, depression of dark respiration, inhibition of net photosynthesis, changing root physiology, membrane structure and so on. But the most visible evidence to show NO's detrimental sides is that gaseous NO obviously inhibited the plants growth, although it didn't induced apparent visible injure at 2 ppm concentration (Wellburn, 1990).

The further research showed that NO involved in photomorphogenesis, mitochondrial activity, leaf expansion, root growth, stomatal closure, senescence, and iron metabolism (Beligni and Lamattina, 2000; Graziano et al., 2002; Leshem et al., 1998; Neill et al., 2002a; Wendehenne et al., 2001). NO is also important for defense response, playing key roles in the activation of defense gene (PR1 and Phytoalexin) and modulation of programmed cell death (PCD) (Delledonne et al., 1998; Durner et al., 1998; Wendehenne et al., 2001). However, NO research in plants is much more in its infancy and the results obtained to date are more intriguing than proofing. The question about its cytotoxic or cytoprotective roles has already been stated (Beligni and Lamattina, 1999a). Because plant NOS and endogenous NO in plants are less known, most experiments are carried out by using animal NOS inhibitors or arbitrary choosing NO concentrations as well as NO sources with possible side effects. It is not surprising that the results from different labs seem to be inconsistent, or even contrary. But due to its multiple and mysterious role, NO has attracted much attention and is becoming an exciting research field in plant biology.

3.1. Biosynthesis of nitric oxide in plants

The presence and synthesis of NO in plant cells is undisputed. The question to be answered is not whether but how do plants produce NO? It was first reported that NO could be produced non-enzymatically. Non-enzymatic NO formation could be the result of chemical reactions between N-oxides and plant metabolites (Cooney et al., 1994), of nitrous oxide decomposition, or of chemical reduction of nitrite (NO_2^-) at acidic pH (Klepper, 1991;

Nishimura et al., 1986). NO is also produced enzymatically from NO_2^- in plants by NAD(P)H-dependent nitrate reductase (NaR) (Yamasaki and Sakihama, 2000). For example, NO production in nonelicited sunflower and spinach leaves appears to be mediated by NaR (Rockel et al., 2002). NaR-mediated NO generation may also play a role in abscisic acid (ABA)-induced stomatal closure in *Arabidopsis* (Desikan et al., 2002). Studies have demonstrated that plant NaRs can convert nitrite to NO under certain conditions *in vitro* (Kaiser et al., 2002; Yamasaki et al., 1999).

In addition to NaR as a possible source for NO, the existence of mammalian-type NOS in plants has been explored since years. NOS-like activity, based on the formation of L-citrulline from L-arginine and/or sensitivity to mammalian NOS inhibitors, has been detected in several plants. Many of the reported NOS activities were Ca^{2+}-dependent, thus resembling mammalian constitutive NOS (Barroso et al., 1999; Cueto et al., 1996; Ribeiro et al., 1999). This hypothesis is further supported by the observation that cryptogein, an elicitor of tobacco defense responses, triggers within minutes an NO burst sensitive to NOS inhibitors in epidermal tobacco cells (Foissner et al., 2000).

In addition, western blot analysis using antibodies raised against mammalian NOSs enabled the detection of immunoreactive proteins in plant extracts. Electron microscopy immunolocalization using such antibodies showed the presence of NOS-like protein in the matrix of peroxisomes and chloroplasts in pea leaves. Moreover, immunofluorescence experiments in maize roots allowed researchers to localize a NOS-like protein in the cytosol of cells in the division zone, and into the nucleus in the elongation zone (Barroso et al., 1999; Ribeiro et al., 1999). However, the confidence of this immunoreaction has been doubted based on the fact that the putative tobacco NOS has a Mr of 56 kD, whereas that from maize is 166 kD (mammalian NOSs range in size from 130-160 kD) (Durner and Klessig, 1999). Recent research showed that using immunological technique is inappropriate to infer the presence of plant NOS. A proteomic approach verified that many NOS-unrelated plant proteins cross-reacted with the mammalian NOS antibodies (Butt et al., 2003).

Although NOS-like activity have been detected in a wide variety of plants and *Arabidopsis* genome has been sequenced, analysis of the complete *Arabidopsis* genome failed to detect a NOS-like gene. The efforts to purify a plant NOS and clone its encoding gene were unsuccessful until very recently. The bottleneck was finally broken by team headed by Daniel F. Klessig (Chandok et al., 2003). Following a six-step purification protocol, and a 33.000-fold purification, biochemical and molecular analysis revealed that the pathogen-inducible nitric oxide synthase (iNOS) in plants is a variant of the P protein of the glycine

decarboxylase (GDC) complex. Since the variant P protein shares very little sequence homology with animal NOSs, this unexpected discovery explains why previous efforts to identify this enzyme failed. Despite the lack of sequence homology, the biochemical properties of the plant enzyme, including cofactor requirements and inhibitor sensitivity, as well as its kinetic properties, appear to be very similar to those of its animal counterparts. Moreover, plant iNOS, like that of animals, is specifically induced during the resistance response to pathogen infection (Chandok et al., 2003).

3.2. NO signaling in plant defense responses

Plants cannot move to escape environmental challenges. Biotic stresses result from a battery of potential pathogens: fungi, bacteria, nematodes and insects intercept the photosynthate produced by plants, and viruses use replication machinery at the host's expense. Plants, in turn, have evolved sophisticated mechanisms to perceive such attacks, and to translate that perception into an adaptive response (Dangl and Jones, 2001).

3.2.1. Mechanisms of plant defense responses

Plants have evolved an array of rapid and efficient defense response against a wide variety of pathogens including bacteria, fungi viruses and nematodes. In many cases, plant defense responses is initiated by a ‘gene for gene’ interaction (Flor, 1971). For example, when a plant has a specific resistance gene (*R*) that interacts with the corresponding avirulence gene (*avr*) from the pathogen, plant can immediately recognize the pathogen and induce a rapid defense mechanism known as programmed cell death (PCD) at the side of pathogen invasion. This ‘hypersensitive response’ (HR) is though to be one of the most powerful weapons and directly kill invaders and /or to interfere with their acquisition of nutrients. But, if either the pathogen or host lacks the corresponding *avr* or *R* gene, then the plant-microbe interaction results in disease (Beers and McDowell, 2001; Dong, 1998). In addition to the localized HR, many plants respond to pathogen infection by activating defenses in uninfected parts of the plant (systemic acquired resistance, SAR). As a result, the entire plant is more resistant to a secondary infection. SAR induction requires the signal molecule salicylic acid (SA), which accumulates in plants prior to the onset of SAR (Sticher et al., 1997; Van Loon, 1999). SAR is believed to be a result of the concerted activation of pathogenesis-related (PR) genes. NPR1

(nonexpresser of PR genes) regulates the PR gene expression that serves as convenient marker for monitoring SAR (Cao et al., 1997; Dong, 1998).

Pathogen-derived *avr* gene products are delivered to intercellular spaces or directly inside plant cells, where they interact with the products of plant *R* genes. The *R* proteins are either transmembrane or intracellular proteins that are presumed to initiate signal-transduction cascades upon ligand binding. Many *R* gene products share structural motifs, indicating that similar pathways might control resistance to diverse pathogens (Dangl and Jones, 2001). To date, over 20 *R* genes have been identified and 5 classes are recognized: (1) intracellular proteins with a nucleotide-binding site (NBS), a leucine-zipper motif and a leucine-rich repeat (LRR) domain; (2) intracellular NBS–LRR proteins with a region of similarity to the cytoplasmic domain of mammalian interleukin-1 receptor (IL-1R) and the *Drosophila* Toll proteins (i.e. the TIR [Toll/IL-1R] domain); (3) intracellular protein kinases (PKs); (4) proteins with an LRR domain that encodes membrane-bound extracellular proteins; and (5) receptor-like kinases (RLKs) with an extracellular LRR domain (Cohn et al., 2001; Dangl and Jones, 2001).

The observation that some *R* gene products share similar amino acid motifs is likely to provide insights into how these proteins interact with downstream signaling components involved in disease resistance. Mammals, insects and plants share common components in signaling pathways of defense response (**Figure 2**). For example, several of the plant *R* genes such as N or Xa21 encode proteins with LRR, DDs and TIR domains, which are found in mammalian interleukin-1 receptors (IL-1R) and the *Drosophila* Toll. Related protein kinases (PK) occur in the defense pathways in all organisms. Owing to the fact that NPR1 shares similarities to I-κB, it has been proposed that NPR1 might act as a negative regulator of gene transcription, similar to mammalian I-κB, which participate in controlling immune responses such as NO production and defensin induction (Despres et al., 2000).

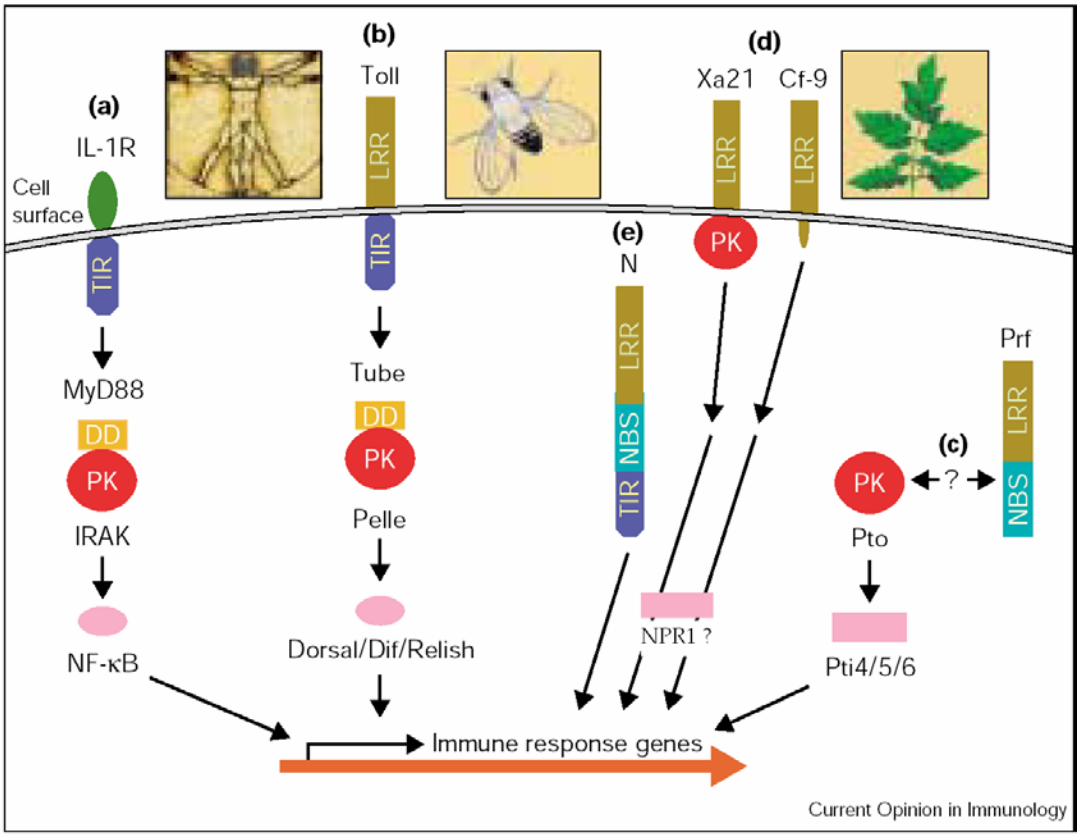


Figure 2: Comparison of the Signaling pathways leading to activation of defense genes in mammals, insects and plants (Figure adapted from Cohn et al., 2001, modified).

3.2.2. The role of NO in plant defense responses

Plants and animals not only share common defense signal components, but possess striking similar weapons against pathogen. Recognition of an avirulent pathogen triggers the rapid production of the reactive oxygen intermediates superoxide (O_2^-) and hydrogen peroxide (H_2O_2). Reactive oxygen species (ROS) are thought to play key roles in defense responses of plant against pathogens. Furthermore, functional homologues of the mammalian NADPH oxidase subunit $gp91^{phox}$ have been identified in several plant species (Simon-Plas et al., 2002). In a manner analogous to their participation in macrophage or neutrophil action, these ROS might be involved in directly killing invading pathogens (Durner et al., 1999). Induction of host cell death and/or induction of defense genes appear to be another line of early plant defense responses that may be triggered by ROS (Alvarez et al., 1998). However, superoxide, the first product of the oxidative burst, is a poor candidate for the cell executioner (at least in plants). Because superoxide has a short half-life, it can rapidly convert to hydrogen peroxide

and does not readily diffuse. Hydrogen peroxide itself is toxic at high concentrations, but not enough of it is commonly produced during resistance responses to kill cells outright. A co-conspirator to PCD maybe exists and has been sought. In mammalian macrophages, ROSs collaborate with NO to execute bacterial pathogens (Nathan and Shiloh, 2000).

In soybean cells, NO potentiates the induction of hypersensitive cell death by reactive oxygen intermediates and functions independently of such intermediates to induce genes for the synthesis of protective natural products. Inhibitors of NOS compromise the hypersensitive disease-resistance response of *Arabidopsis* leaves to *Pseudomonas syringae*, promoting disease and bacterial growth. These finding suggest that NO plays a key role in disease resistance in plants (Delledonne et al., 1998). Moreover, tobacco was infected with resistant, but not susceptible tobacco mosaic virus resulted in enhanced NO synthase (NOS) activity. Furthermore, NO donors or recombinant mammalian NOS triggered expression of pathogenesis-related protein (PR1) and phenylalanine ammonia lyase (PAL) in tobacco plants or tobacco suspension cells. These genes were also induced by cyclic GMP (cGMP) and cyclic ADP-ribose, two molecules that can serve as second messengers for NO signaling in mammals. Consistent with cGMP acting as a second messenger in tobacco, NO treatment induced dramatic and transient increases in endogenous cGMP levels. Furthermore, NO-induced activation of *PAL* was blocked by two inhibitors of guanylate cyclase (Durner et al., 1998). Recently, a pathogen inducible nitric oxide synthase (iNOS) was purified in tobacco and its corresponding gene in *Arabidopsis* was cloned (Chandok et al., 2003). Many other publications also demonstrate the participation of NO, peroxynitrite and cGMP in plant apoptosis, gene regulation and defense responses against pathogens (Alamillo and García-Olmedo, 2001; Clark et al., 2000; Clarke et al., 2000; Clough et al., 2000; Shirasu and Schulze-Lefert, 2000; Wendehenne et al., 2001; Wojtaszek, 2000; Yamasaki, 2000).

Summarily, nitric oxide (NO)-mediated signaling pathway in plant defense response could be proposed as in **Figure 3** (Wendehenne et al., 2001). Recognition of an infecting pathogen leads to the production of reactive oxygen species (ROS such as superoxide [$O_2^{\cdot}$-] and hydrogen peroxide [H_2O_2]) possibly by NADPH oxidase and NO by NOS. ROS and NO collaborate to execute pathogens via formation of peroxynitrite (ONOO-), which is highly toxic and could play an important role in the activation of the cell death program. In another pathway, NO might convert cytosolic aconitase into an iron regulatory protein (IRP), which modulates the translation and stability of mRNAs encoding proteins involved in the intracellular iron homeostasis (Navarre et al., 2000). The resulting increase in free iron concentration promotes the Fenton reaction, leading to formation of the hydroxyl radical HO$^{\cdot}$,

which creates a killing environment for both host and pathogen. NO could also affect ethylene production during pathogen attack.

As already mentioned, NO effects transcriptional activation of defense genes. This model suggests salicylic acid-dependent (e.g. PR-1) as well as salicylic acid-independent gene (e.g. PAL) induction. For some genes, full activation might require the simultaneous presence of both cADPR and cGMP (Wendehenne et al., 2001). However, which defense genes are NO-dependent and how does NO interact with other known defense signals (e.g. SA and jasmonic acid) are still less known.

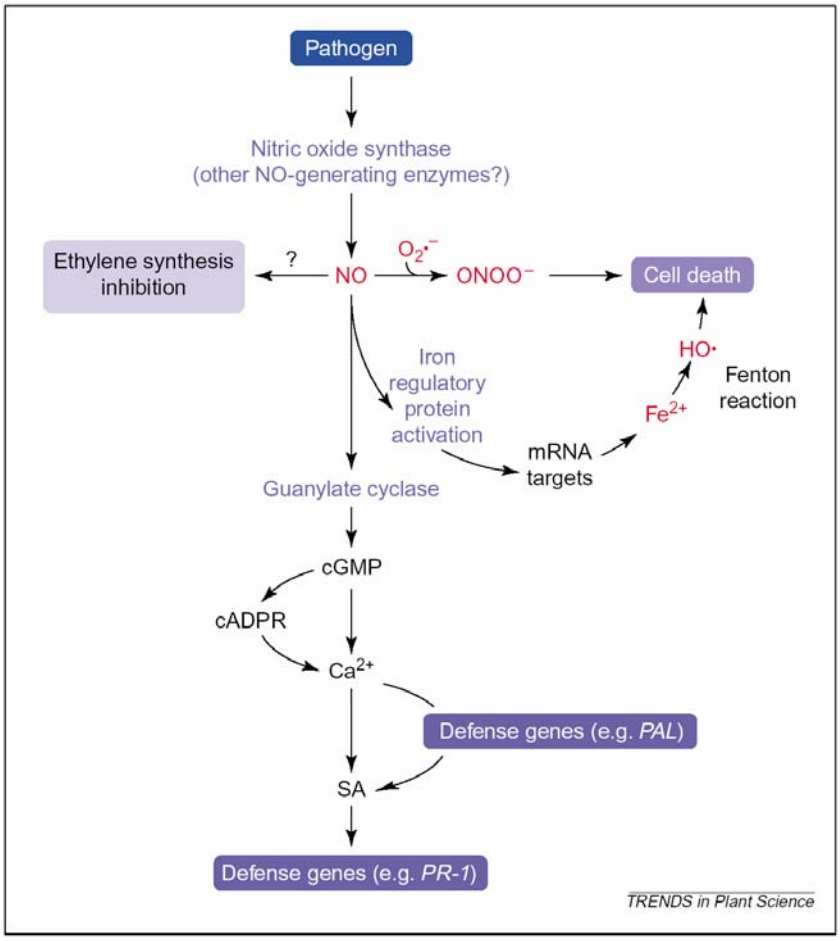


Figure 3: Nitric oxide (NO) mediated signaling pathway in plant defense against pathogen (Figure adapted from Wendehenne et al., 2001).

4. Goal of the work and the research strategy

Briefly, the goals of this work were:

1. Identifying NO-responsive genes by microarray analysis.
2. Verifying and explaining the induction/suppression of genes of interest within their biological context.

4.1. Simulation of NO burst in *Arabidopsis* plants and suspension cells

Arabidopsis has been selected as research model plant, because the genome of *Arabidopsis* is relative small and has recently been completely sequenced, which enables us to a comprehensive genome-wide survey of gene expression patterns. The expression pattern can be viewed as maps that reflect the order and logic of the genetic program, rather than the physical order of genes on chromosomes. I carry out the research in two systems: whole plant systems and cell suspension systems. Suspension cells are a good model to study intracellular, but not intercellular signaling. To study NO intercellular signaling, transcript analysis in whole plant is necessary.

The origin of NO in plants and the concentrations, at which it exerts its functions, is hitherto still not known. To study the NO dependant gene induction, the best strategy is to simulate the NO burst in plants as well as in cell suspension. Treatment with NO donors is a favorite strategy of NO simulation in cell culture. But as we work with NO donors, we have to keep in mind that we are working with side effects. Side effects cannot be avoided, but can be reduced. What I can do is to use NO donors with fewer side effects. (+/-)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide (NOR3) is a novel NO donor, which has been isolated from microbial products and reported to release NO spontaneously and stoichiometrically 1-1.5 mol /mol of NOR3 in physiological pH (Kita et al., 1994). Compared to other classical NO donors, such as sodium nitroprusside (SNP) or *S*-nitroso-*N*-acetylpenicillamine, NOR3 is assumed to be superior in the present kinetic studies. NOR3 is known to be an excellent NO donor to study NO-signaling both in the animal and the plant field, especially for treatment of cell layers and/or cell suspensions (Yamamoto et al., 2000). In addition, to analyze NO-mediated defense gene expression in *Arabidopsis* plants, and to obtain large quantities of uniformly treated material, whole plants were treated with gaseous NO.

4.2. Use of cDNA microarrays to study different gene expression

Various methods are available for detecting and quantitating gene expression levels, including northern blots (Alwine et al., 1977), S1 nuclease protection (Berk and Sharp, 1977), differential display (Liang and Pardee, 1992), sequencing of cDNA libraries (Adams et al., 1991; Okubo et al., 1992), serial analysis of gene expression (SAGE) (Velculescu et al., 1995) and cDNA microarrays.

The most common use of these is for the determination of patterns of differential gene expression, comparing differences in mRNA expression levels between identical cells subjected to different stimuli or between different cellular phenotypes or developmental stages. Microarray expression analysis (Schena et al., 1995) has a number of features that have made it the most widely used method for profiling mRNA expression. DNA segments representing the collection of genes to be assayed are amplified by PCR and mechanically spotted at high density on glass microscope slides using relatively simple *x-y-z* stage robotic systems, creating a microarray containing thousands of elements. Microarrays containing the entire set of genes from a microbial genome or tens of thousands of eukaryotic cDNA clones can be easily constructed. The microarrays are queried in a co-hybridization assay using two or more fluorescently labeled probes prepared from messenger RNA from the cellular phenotypes of interest (Shalon et al., 1996). The kinetics of hybridization allows relative expression levels to be determined based on the ratio with which each probe hybridizes to an individual array element. Hybridization is assayed using a confocal laser scanner to measure fluorescence intensities, allowing simultaneous determination of the relative expression levels of all genes represented in the array.

Currently, several companies offer *Arabidopsis* genome covering arrays. The commercial arrays are expensive. At the first stage, the fluorescent labeling protocol using 330 stress and/or redox-regulated genes that were previously developed in institute of biochemical plant pathology (BIOP) should be established. Once the standard protocol is proved to be economic and efficient, the array is extended by increasing the number of genes to be analyzed.

4.3. Verify and explain the gene induction

Microarray analysis should reveal which gene can be activated or suppressed by NO. This could help us to insight the role of NO in the plant signaling network and to define the

downstream components of NO signaling. In addition, I wanted to verify the microarray results by classical methods (e.g. northern blot) and follow the hints obtained through expression profiling to carry out further research, possibly using biochemical and physiological methods. Moreover, I wanted to elucidate the effects of NO in plant defense response, especially the interaction between NO and other known defense signals (e.g. SA, JA and ethylene). To reveal this interaction, transcripts of wild type plants were compared with that from mutants/transgenes impaired in plant defense responses.

Thus, the data generated by these approaches should eventually increase our understanding of NO's role in plant defense responses, and they should help to define NO's place within the complex signaling pathways.

II. Materials and Methods

1. Materials

1.1. Plant materials

Abbr. Name	Species	Eco. type	Function	Provenance	Reference
col. wt	*Arabidopsis thaliana*	Columbia	Wild type		
etr1	*Arabidopsis thaliana*	Columbia	Ethylene resistant mutant	D. Ernst	(Chang et al., 1993)
jin1	*Arabidopsis thaliana*	Columbia	Jasmonic acid insensitive mutant	S. Berger	(Berger, 2002)
dad1	*Arabidopsis thaliana*	Columbia	Defense, no death	A. Bent	(Yu et al., 1998)
sai	*Arabidopsis thaliana*	Nossen	Salicylic acid insensitive mutant	D. F. Klessig	(Shah et al., 1997)
jar1	*Arabidopsis thaliana*	Columbia	Jasmonic acid resistant mutant	S. Berger	(Berger, 2002)
NahG	*Arabidopsis thaliana*	Columbia	Expression of bacterial salicylate hydroxylase gene	D. F. Klessig	(Gaffney et al., 1993)
opr3	*Arabidopsis thaliana*	Columbia	JA synthesis mutant	J. Browse	(Stintzi and Browse, 2000)
pad4	*Arabidopsis thaliana*	Columbia	Phytoalexin deficient mutant	NASC	(Glazebrook et al., 1997).
tobacco	*Nicotiana tabacum*		Wild type		

1.2. Chemicals

Name	Company
aa-dUTP (5-(3-aminoallyl)-2'deoxyuridine-5'-triphosphate)	Sigma, Deisenhofen
Agarose	Biozeym, Oldendorf
Ampicillin	Sigma, Deisenhofen
BAP (6-Benzylaminopurin)	Serva, Heidelberg
Betaine (Monohydrate)	Sigma, Deisenhofen

Bromphenol blue	Sigma, Deisenhofen
BSA (Bovine Albumin)	Sigma, Deisenhofen
Chloroform	Merck, Darmstadt
Cy-3 ester	AmershamPharmacia, Buckinghamshire, UK
Cy-5 ester	AmershamPharmacia, Buckinghamshire, UK
DAF-2 DA (4,5-Diaminofluorescein diacetate solution)	Alexis, Lausen, Switzerland
DEPC (Diethyl cyanophosphonate)	Sigma, Deisenhofen
DETC (Sodium diethyldithiocarbamate hydrate)	Sigma, Deisenhofen
DMSO (methyl sulfoxide)	Sigma, Deisenhofen
cPTIO (2-(4-carboxypheneyl)-4,4,5,5-tetramethylimidazoline-1-1oxyl-3-oxide.potassium salt)	Alexis, Lausen, Switzerland
EDTA (Ethylenediaminetetraacetic acid)	USB, Ohio, USA
Ethidium bromide	Sigma, Deisenhofen
Ficoll (Type 400)	AmershamPharmacia, Buckinghamshire, UK
Formaldehyde	Sigma, Deisenhofen
Formamide	Sigma, Deisenhofen
Glycerin	Roth, Karlsruhe
HEPES (N-2-hydroxyethyl-piper-az-ine-N'-2-ethane-sulphonate)	Sigma, Deisenhofen
Isopropanol	Merck, Darmstadt
Jasmonic acid	Sigma, Deisenhofen
MOPS (4-Morpholinepropanesulfonic acid; 3-Morpholinopropanesulfonic acid)	Sigma, Deisenhofen
NOR3 ((+/-)-(E)-4-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide)	Alexis, Lausen, Switzerland
Polyvinylpyrrelidon (PVP-360)	Sigma, Deisenhofen
RNaseZAP	Sigma, Deisenhofen
SHAM (salicylhydroxamic acid)	Sigma, Deisenhofen

Sodium acetate	Sigma, Deisenhofen
Sodium Borohydride	Sigma, Deisenhofen
Sodium citrate	Sigma, Deisenhofen
ssDNA (Salmon sperm DNA)	AmershamPharmacia, Buckinghamshire, UK

1.3. Molecular biological Kits

CSPD solution	Roche, Mannheim
DIG antibody	Roche, Mannheim
DIG Easy Hyb Granules	Roche, Mannheim
DIG Wash and Block Buffer Set	Roche, Mannheim
PCR DIG Probe Synthesis Kit	Roche, Mannheim
Qiagen Plasmid Mini Kit	Qiagen, Hilden
QIAquick PCR Purification Kit	Qiagen, Hilden
Superscript^{TM} II RNase H^{-} Reverse Transcriptase Kit	Invitrogen, Eggenstein
Taq-polymerase	pebLab, Erlangen
TRIzol® reagent	Invitrogen, Eggenstein

1.4. Buffers and solutions

aa-dNTP Mix (50X)	dATP dCTP dGTP dTTP aa-dUTP	25 mM 25 mM 25 mM 15 mM 10 mM
Ampicillin stock solution	Ampicillin Sterile filter and store at -20°C	25 mg/ ml in H_2O
DEPC-treated ddH_2O	0.01% (V/V) diethylpyrocarbonate (DEPC) was added in water in RNase-free glass bottles. Let it stand overnight and autoclave.	

Denhardt solution (100×)	Ficoll Polyvinylpyrrelidon BSA Add H_2O to Sterile filter and store at -20°C	10 g 10 g 10 g 500 ml
DNA extraction buffer	Hexadecyltrimethyl-ammonium bromide (CTAB) NaCl EDTA Tris (pH8.0) PVP 40 Ascorbic acid Diethyldithiocarbamic acid Autoclave	2% (w/v) 1.42 M 20 mM 100 mM 2% (w/v) 5 mM 4 mM
Hybridization buffer (for microarray)	Formamide 20×SSC 10% SDS 100×Denhardt solution ssDNA (10 mg/ ml) Add H_2O to	50 ml 30 ml 5 ml 5 ml 1 ml 100 ml
MOPS Buffer (10×)	MOPS Sodium acetate EDTA Adjust to pH 7.0	200 mM 50 mM 10 mM
PCR buffer (10×)	Tris $(NH_4)_2SO_4$ $MgCl_2$ Tween 20 Adjust with HCl to pH 9.0	750 mM 200 mM 15 mM 0.1% (v/v)
Phosphate buffer (1M)	1M K_2HPO_4 1M KH_2PO_4 Adjust to pH 8.5	9.5 ml 0.5 ml
Phosphate elution buffer (for aa-dUTP labeling cDNA synthase)	1 M KPO_4 Add H_2O to Adjust to pH 8.5	4 ml 1000 ml
Phosphate wash buffer (for aa-dUTP labeling cDNA synthase)	1 M KPO_4 MilliQ water 95% ethanol Total	0.5 ml 15.25 ml 84.25 ml 100 ml

Phosphate-buffered saline (PBS)	NaCl KCl Na_2HPO_4 KH_2PO_4 Adjust to pH 7.4	137 mM 2.7 mM 10.0 mM 2.0 mM
Pre-hybridization buffer (for microarray)	20×SSC 10% SDS BSA ssDNA (10 mg/ ml) Add H_2O to	30 ml 5 ml 1 g 1 ml 100 ml
RNA probe buffer	Formaldehyde Formamide deion. 10×MOSP buffer Bromphenol blue	30 µl 8700 µl 500 µl trace
SSC (20×)	NaCl Sodium citrate Adjust to pH 7.0	3 M 0.3 M
ssDNA stock solution	Salmon sperm DNA Dissolve the ssDNA in water, autoclave, and store at -20°C. Before adding to hybridization buffer, ssDNA was denatured at 100°C for 2 min and cooled on ice.	10 mg/ ml
TE buffer	Tris EDTA Adjust to pH 8.0	10 mM 1 mM
TBE buffer	Tris-borate EDTA Adjust to pH 8.0	45 mM 1 mM
TRIzol Reagent	Phenol in saturated buffer Guanidine thiocyanate Ammonium thiocyanate Sodium acetate, pH5 Glycerol Add H_2O to	38 % 0.8 M 0.4 M 0.1 M 5 % 1.0 liter

1.5. Mediums

LB glycine medium	K_2HPO_4	36.0 mM
	KH_2PO_4	13.2 mM
	$MgSO_4$	0.4 mM
	Natrium citrate	1.7 mM
	$(NH_4)_2SO_4$	6.8 mM
	Glycine	4.4%
	Tryptone	1% (w/v)
	Yeast extracts	0.5% (w/v)
	NaCl	1% (w/v)
	Adjust to pH 8.0	
PS medium	MS Macro element (10×)	100 ml
	MS Micro element (1000×)	1 ml
	MS Vitamin	10 ml
	Fe-EDTA (200×)	5 ml
	2, 4-D (2 mg/ ml)	1 ml
	Saccharose	30 g
	Agar	12 g
	BAP (1 mg/ ml)	1 ml
	Add H_2O to	1000 ml
	Adjust to pH 6.0	
	Autoclave	

Stock solutions of PS medium:

MS macro element (10×)	NH_4NO_3	33.0 g
	KNO_3	38.0 g
	$CaCl_2 .2H_2O$	8.8 g
	$MgSO_4 .7H_2O$	7.4 g
	KH_2PO_4	3.4 g
	KI	16.6 mg
	Add bidist. H_2O to	2000 ml
	Autoclave	
MS micro elements (1000×)	$MnSO_4 .H_2O$	3340 mg
	H_3BO_3	1240 mg
	$ZnSO_4 .7H_2O$	2120 mg
	$Na_2MoO_4 .2H_2O$	50 mg
	$CuSO_4 .5H_2O$	50 mg
	$CoCl_2 .6H_2O$	50 mg
	Add H_2O to	200 ml

MS Vitamins (100×)	Nicotinic acid Pyridoxine-HCl Thiamine-HCl m-Inosite Add H_2O to Store at -20°C	25 mg 25 mg 5.3 mg 5000 mg 500 ml
Fe- EDTA solution (100×)	$FeSO_4$ **.**$7H_2O$ Na_2 EDTA **.**$2H_2O$ Dissolve respectively in 200 ml H_2O and slowly mix together, add H_2O up to 500 ml and warm up to 60°C until turning yellow.	2.78 g 3.72 g

1.6. Apparatus

Alpha 1-5 freeze-dryer	Christ, Osterode
Array robot GMS 417	Genetic Microsystems
AXON GenePix 4000 scanner	Axon, CA, USA
Brucker ESP300 X-band spectrometer	Brucker, Karlsruhe
Centrifuge 5415D	Eppendorf, Hamburg
Centrifuge LL Universal	Hittich, Tuttingen
Clark-type oxygen electrode cuvette	Hansatech, Bachofer
HybChamber^{TM}	GeneMachines, California, USA
MicroGrid II Arrayer	Apogent Discoveries, UK
Milli-Q Water System	Millipore, Eschborn
PCR thermocycler	Hybaid, Heidelberg
pH-Meter 743	Knick, Berlin
UV-Crosslinker Stratalinker	Stratagene, Heidelberg
Vacuum manifold filtration system	Millipore, Eschborn
Zeiss LSM 510 confocal laser microscope	Zeiss, Oberkochen

1.7. Consumed materials

96-well microtiter plate	Nalge Nunc, Naperville, USA
96 well multiscreen filter plates	Millipore, Eschborn
96 well reaction plates	ABgene, Surrey, UK
382-well microtiter plate	Greiner, Oberschleißheim
ABgene Adhesive PCR Film	ABgene, Surrey, UK
AirPore™ Tape Sheets	Qiagen, Hilden
CSS-100 silylated Slides	CEL Associates, Houston
Eppendorf- reaction tube	Eppendorf, Hamburg
Falcon tube	Becton Dickinson labware, NJ, USA
Hybridization bag	Life Technologie, Galthersburg, USA
Lumi-film chemiluminescent detection film	Roche, Mannheim
Microarray Gene Frame®	ABgene, Surrey, UK
Nylon Membranes, positively charged	Roche, Mannheim
Tape Pads	Qiagen, Hilden

2. Methods

2.1. Growth condition of *Arabidopsis* plants and treatment with NO

Seeds from plants (*Arabidopsis thaliana*, ecotype Columbia or, when indicated, ecotype Nössen) were sown on potting compost and vernalized for 2 days at 4°C. Plants were grown for 4 to 5 weeks in a growth chamber (at 69% relative humidity, 10 hr light) at 23°C during the day and 18°C at night.

The experimental setups to study the effect of NO on whole plants consisted of controlled environment cabinets as well as complete instrumentation to adjust and control gaseous NO through an electrochemical sensor. In experiments, *Arabidopsis* plants were treated with NO concentrations of 1250 ppm for 10 min. At this NO concentration, the plants did not show any symptoms. After this treatment, the plants were maintained in growth chambers and harvested at different time. Leaf material was quickly frozen in liquid nitrogen and stored at -80° until further use.

2.2. Cell culture and NO treatment

Cell suspensions were cultured in liquid PS-medium (see 1.2.4.) at 27°C and stirred at 120 rpm in darkness. Cell culture was transferred to a new PS-medium weekly. A 7 days old cell suspension was treated with the NO donor NOR3 ((E)-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexene-amide) at final concentration of 0.5 mM. After treatment, cells were harvested by filtration at various times. The cells were quickly frozen in liquid nitrogen and stored at -80°C until further use.

2.3. Microarray

For the analysis of expression in most eukaryotes, expressed sequence tag (EST) data represent the most extensive data for gene identification. ESTs are single-pass, partial sequences of cDNA clones, and they have been used extensively for gene discovery in many organisms. The EST approach has been widely adopted; more than 71% of all GenBank entries and 40% of the individual nucleotides in the database are derived EST sequences (Schuler, 1997). 330 EST of so called “stress genes” defense-related genes encoding PR-

proteins or proteins induced by pathogens, oxidative, cold, UV, ozone, or heavy metal stress. (See **Supplement Table S1**) were assembled in BIOP by work groups headed by PD Dr. Anthon Schaeffner and Dr. Dietrich Ernst. Most of them are cDNA clones (expressed sequence tags [ESTs]) obtained from the *Arabidopsis* Biological Resource Center (Columbus, OH). Some of them were cloned by Dr. Bern Heidenreich in his doctoral work (Heidenreich et al., 2001). These genes were chosen for transcripts analysis after NO treatment.

2.3.1 PCR amplification and purification of target DNA

2.3.1.1 Amplification of the target DNA

cDNA clone inserts were amplified by PCR from plasmid DNA or directly from clones in culture. In high-throughput applications, amplification of clones from culture has the advantage of being both more cost efficient and less labor intensive with lower cross-contamination rates than amplification from plasmid DNA. Clone inserts were amplified using the following protocol:

A. Selected clones were inoculated in 96 well microtiter plate containing 100µl LB-glycerol medium with ampicillin (100µg/ ml). Microtiter plates were covered with AirPore^{TM} Tape Sheets and incubated for 16 hours at 37ºC. The bacteria suspension were directly used for PCR or stored for future use at -80°C. Alternatively, plasmid DNAs were isolated by using Qiagen Plasmid Mini Kit and 1:50 diluted with water. The diluted plasmid DNA served as PCR template.

B. Clone inserts were amplified in 100µl PCR reactions in 96 well reaction plates. A reaction master mix was prepared for each reaction plate:

10×complete PCR buffer (with 15 mM $MgCl_2$)	1000 µl
M13 Forward primer (10µM)	300 µl
M13 Reverse primer (10µM)	300 µl
dNTP mix (10 mM per dNTP)	300 µl
Taq-polymerase (5U/µl)	50 µl
MilliQ water	7950 µl
Total	9900 µl

The amplification primers were M13 primers with C6 amino modification to the 5' end (purchased from MWG, Ebersburg) with the following sequences:
M13 FWD: 5'- GTA AAA CGA CGG CCA GT -3'
M13 REV: 5'- GGA AAC AGC TAT GAC CAT G- 3'

C. For each reaction, 2µl of culture supernatant or 2 µl of diluted plasmid solution were added to 98µl of master mix in 96 well PCR reaction plate with multi-pipette. The PCR reaction plate was covered with ABgene Adhesive PCR Film.

D. Reactions were amplified in a Hybaid thermocycler using the following cycling program:

95°C	5 min		initial denaturizing
95°C	30 sec	30 cycles	denaturizing
52°C	30 sec		annealing
72°C	2 min		extension
72°C	5 min		additional extension
4°C forever			

E. 6 µl of PCR product were mixed with 1.5 µl of DNA loading Buffer and then loaded in 1% agarose gel, and electrophoresed in 1×TBE buffer for 2hr to test the PCR products.

2.3.1.2. Purification of target DNA

For efficient binding of the amplified clone inserts to the slides, it is essential to remove unincorporated nucleotides and primers from the reaction products. 96 well multiscreen filter plates were used to purify PCR products. PCR products were cleaned using the following filtration protocol:

A. 200 µl PCR product were transferred to the Millipore filter plate.

B. Filter plate was placed on a Millipore vacuum manifold filtration system and filtered at a pressure of 15in (380 mM) Hg for 10 minutes or until the plate dry.

C. 50µl MilliQ water was added to each well and filtered at 15mm (380 mM) Hg for 5-10 minutes or until the plate dry.

D. Step C was repeated once.

E. Filter plate was removed from the manifold filtration system. 50µl of MilliQ water were added to each well. Filter plate was placed on a shaker and shaked vigorously for 10 minutes to resuspend the DNA.

F. The purified PCR products were manually pipetted into a new 96 well plate.

G. Plates containing the purified PCR products were then sealed using a Qiagen Tape Pads and stored at 4°C for future arraying.

2.3.2 Array printing

50 µl purified amino-modified PCR products were dried by lyophylization in a Christ Alpha 1-5 freeze-dryer and suspended in 20 µl spotting solution (3×SSC supplemented with 1.5M betaine). Using betaine can not only reduce evaporation, but improve the binding efficiency and the homogeneity of spotted DNA (Diehl et al., 2001). PCR products were arrayed from 384-well microarray plates onto silylated microscope slides using a MicroGrid DNA arraying robot. The printing robot is a x, y, z motion control unit equipped with a printing head containing printing tips. The tips are dipped into the samples and then moved towards the slides, where they touch the surface and deliver a small aliquot forming a dot with a diameter of ca 0.1 mM. The coupling reaction of amino-modified DNA to aldehyde-coated surface was shown in **Figure 4**.

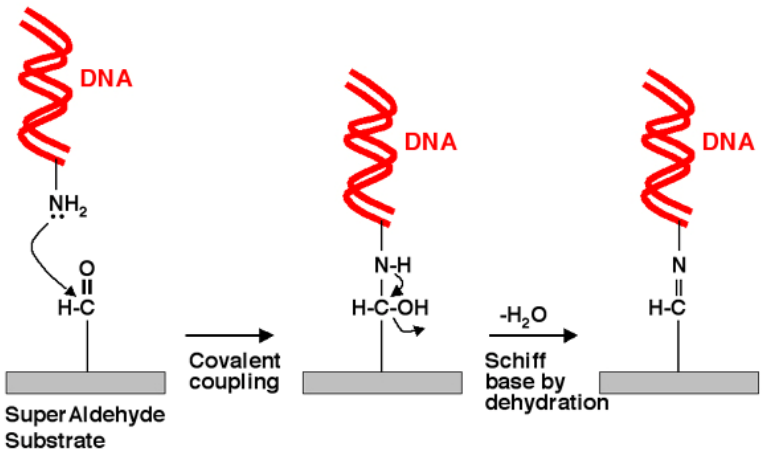


Figure 4: The coupling reaction of amino-modified DNA to aldehyde-coated surface (Figure adapted from http://www. arrayit.com/Products/ Substrates/SMA/sma.ht ml #CPAld). Primary amino linkers (NH2) on the DNA (red ribbons) attack the aldehyde groups (left panel) forming covalent bonds (center panel). Attachment is stabilized by a dehydration reaction (drying in low humidity), which leads to Schiff base formation (right panel). Specific and covalent end attachment provides highly stable and accessible attachment of DNA for gene expression and genotyping applications.

2.3.3. Post-washing and blocking

Following processes are indispensable to remove unbound material, and block unreacted aldehyde groups to reduce non-specific binding of labeled reactants (Schena et al., 1996).

A. The spotted substrates were kept in solution form for at least one day and dried by lyophilization.
B. The printed substrates were rinsed twice in 0.1% SDS and once in dH_2O for 2 min at room temperature (22-25°C) to remove unbound DNA.
C. The Substrates were rinsed once in dH_2O for 2 min at room temperature with vigorous agitation.
D. Treatment with sodium borohydride ($NaBH_4$) reduces unreacted aldehyde groups to alcohols, which lowers fluorescent background in assays. For sodium borohydride reduction, the slides were immersed in sodium borohydride reducing solution [0.75g $NaBH_4$ were dissolved in 200 ml phosphate buffered saline (PBS), 75 ml 100% ethanol were added to reduce the bubbles], and then reduced for 5 min with gentle mixing.
E. The slides were transferred into boiling dH_2O at 100°C for 2 min to denature the DNA.
F. The slides were rinsed twice for 1 min in 0.1% SDS.
G. The slides were rinsed twice for 1 min in 500 ml dH_2O, and dried the processed substrates by centrifugation for 1 min at 500x g in a centrifuge (Hettich, Model LL Universal).

2.3.4 Probe labeling

2.3.4.1 RNA extraction

Microarray assay differential gene expression by co-hybridization of fluorescent labeled probes prepared from different RNA sources. As with many other RNA-based assays, the purity and quality of the starting RNA has a significant effect on the results of the assay. Further, the products of the labeling reactions must be cleaned to remove unincorporated labeled nucleotides that can produce a significant background on the substrate following hybridization. I have found that TRIzol reagent gives consistently high quality RNA from cell culture and many tissue samples. TRIzol extraction is quick and produces a high yield of total RNA.

A. Homogenization

Ca. 100 mg tissue was homogenized in a plastic Eppendorf centrifuge tube containing 1 ml TRIzol reagent. The tubes were shaked vigorously by hand and incubated for 5 min at room temperature to permit the complete dissociation of nucleoprotein complexes.

B. Phase separation

0.2 ml of chloroform per 1 ml of TRIzol Reagent was added. Tubes were capped securely, and shaked vigorously with vortex for 15 sec, and then incubated at room temperature for 2 to 3 minutes. Following centrifugation at 12,000×g for 15 minutes at 4°C, the mixture separates into a lower red, phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase.

C. RNA precipitation

Aqueous phase was carefully pipetted into a clean Eppendorf centrifuge tube. Interphase and lower phase were discarded. The RNA was precipitated from the aqueous phase by mixing 0.5 ml of isopropyl alcohol per 1 ml of TRIzol Reagent used for initial homogenization. The mixed solution was incubated at room temperature for 10 min, and then centrifugated at 12,000×g at 4°C for 15 min.

D. RNA wash

The supernatant was discarded. Pellet was washed with 1 ml of 75% ethanol by vortex briefly. The tube was centrifugated at no more than 7,500×g at 4°C for 5 min. Supernatant was discarded. The pellet was air dried for ca. 10 min and resuspended in 50 µl DEPC-H_2O by pipetting up and down a few times. The suspension was incubated at 55 -60 °C for 10 min to dissolve the pellet completely. An aliquot was put into the spectrophotometer to determine RNA concentration and quality. For optimal spectrophotometric measurements, RNA aliquotes were 1:35 diluted with DEPC- H_2O.

2.3.4.2. cDNA synthesis

Probes for microarray analysis were prepared from RNA templates by incorporation of fluorescently labeled deoxyribonucleotides during first strand cDNA synthesis. Either total or poly(A^+) RNA can be used in the reverse transcription reaction, but oligo (dT) labeling of total RNA provides consistently high-quality probes from smaller quantities of starting RNA and without the expense of poly(A^+) purification. I prepared labeled probes using indirect aminoallyl labeling method, namely, cDNA synthesis and fluorescent labeling were divided (Protocol was adapted from http://pga.tigr.org/sop/M004-1a.pdf, modified). This method was proved to be high efficient and lower costing. cDNA were synthesized using the following protocol:

A. 4 µl Poly-$d(T)_{12-18}$ primers (0.5 mg/ ml) were added to 20µg of total RNA and the final volume was adjusted up to 18.5 µl with RNase-free water.

B. The sample was well mixed by vortex and incubated at 70°C for 10 minutes, snap-frozen in ice for 30 seconds, and then centrifugated briefly.

C. A reaction master mix with following components was added and mixed by vortex.

5X First Strand buffer	6 µl
0.1 M DTT	3 µl
10X aminoallyl-dNTP (aa-dNTP) mix	3 µl
SuperScript II RT (200U/ µl)	2 µl

D. The tube was incubated at 42°C for 3 hours to overnight.

E. To hydrolyze RNA, 10 µl of 1 M NaOH and 10 µl of 0.5 M EDTA were added and incubated at 65°C for 15 minutes.

F. 10 µl of 1M HCl was added to neutralize pH.

2.3.4.3. Reaction Purification I: Removal of unincorporated aminoallyl-dUTP (aa-dUTP) and freeamines

This purification protocol was modified from the Qiagen QIAquick PCR purification kit protocol. The phosphate wash and elution buffers (see **II 1.4.)** were substituted for the Qiagen supplied buffers because the Qiagen buffers contain free amines which compete with the Cy dye coupling reaction.

A. aa-dUTP modified cDNA was mixed with 300 µl (5X reaction volume) buffer PB (Qiagen supplied) and transfer to QIAquick column.

B. The column was placed in a 2 ml collection tube (Qiagen supplied) and centrifuged at ~13,000 rpm for 1 minute. Collection tube was emptied.

C. 750 µl phosphate wash buffer were added to the column and centrifuged at ~13,000 rpm for 1 minute.

D. The collection tube was emptied and step (C) was repeated once.

E. The collection tube was emptied and the column was centrifuged for an additional 1 minute at maximum speed.

F. The column was transferred to a new 1.5 ml microfuge tube and 30 µl phosphate elution buffer (see **II 1.4.**) were carefully added to the center of the column membrane.

G. Incubated for 1 minute at room temperature.

H. The cDNA was eluted by centrifugation at ~13,000 rpm for 1 minute.

I. The cDNA was eluted for a second time by repeating steps (F).

J. The final elution volume should be ~60 µl.

K. The sample was dried in a speed vac.

2.3.4.4. Coupling aminoallyl-labeled cDNA to Cyanine dye ester

NHS-ester Cyanine 3 dye (Cy3) and NHS-ester Cyanine dye Cyanine 5 (Cy5) are provided as a dried product in 5 tubes. A tube of dye ester was resuspended in 73 µl of DMSO before use. The reaction tubes was wrapped with foil and kept covered as much as possible in order to prevent photo bleaching of the dyes. Dye esters were either used immediately or aliquoted and stored at -80°C. Any introduced water to the dye esters will result in a lower coupling efficiency due to the hydrolysis of the dye esters. Since DMSO is hygroscopic (absorbs water from the atmosphere), it is well sealed in desiccant.

Aminoallyl-labeled cDNA was resuspended in 4.5 µl of 0.1 M sodium carbonate buffer (Na_2CO_3), pH 9.0 (Carbonate buffer changes composition over time, it was made fresh every couple of weeks to a month), and then added 4.5 µl of the NHS-ester Cy dye. To prevent photo bleaching of the Cy dyes, all reaction tubes were wrapped in foil and kept sequestered from light as much as possible. The reaction was incubated for 1 hour in dark at room temperature and mixed by vortex briefly every 10 min.

2.3.4.5. Reaction purification II: removal of uncoupled dye

The uncoupled dye was removed by using Qiagen QIAquick PCR purification kit. This protocol is modified from the Qiagen QIAquick Spin Handbook (04/2000, pg. 18).

A. 35 µl 100 mM NaOAc (pH 5.2) were mixed with the dye labeled cDNA and transferred to a QIAquick spin column.
B. 250 µl PB buffer were added to the column.
C. The column was placed in a 2 ml collection tube and centrifuged at ~13,000 for 1 minute. The collection tube was emptied.
D. To wash, 0.75 ml buffer PE were added to the column and centrifuged at ~13,000 for 1 minute.
E. The column was placed in a clean 1.5 ml microfuge tube. 30 µl Buffer EB were added to the center of the column membrane.
F. Incubated for 1 minute at room temperature.
G. The cDNA was eluted by centrifugation at ~13,000 rpm for 1 minute.
H. The cDNA was eluted for a second time by repeating steps (E).
I. The final elution volume should be ~60 µl.
J. The sample was dried in a speed vacuum.

2.3.5. Pre-hybridization

The goal in any hybridization is to obtain high specificity while minimizing background. Aldehyde slides bind DNA with high efficiency. Non-specific binding is prone to happen, albeit, the free aldehyde groups on the slide have been inactivated chemically prior to hybridization. Non-specific binding to slide would not only deplete cDNA, but enhance background. Pre-hybridization in a solution containing 1% bovine serum albumin can effectively eliminate nonspecific binding of the probe to the slide. Pre-hybridization has the additional advantage of washing unbound DNA from the slide prior to the addition of the probe. Any DNA that washes from the surface during hybridization competes with DNA bound to the slide. As the kinetics of solution hybridization is much more favorable than surface hybridization, this can dramatically decrease the measured fluorescence signal from the microarray.

The slides were immersed into the pre-hybridization buffer (see **II 1.4.**) at 42°C for 45 min, and then washed with water thoroughly, and then air-dried. Slides were used immediately following pre-hybridization. It was found that hybridization efficiency decreases rapidly if the slides were allowed to dry for more than one hour (Hegde et al., 2000).

2.3.6. Hybridization

The purified aa-dUTP labeled cDNA pair (control and treatment) were combined together and dried by speed vacuum, and then resuspended with 100 µl hybridization buffer. Double-stranded probe was denatured by heating for 2 min at 95°C, and then transferred to ice. The supernatant was pipetted on the pre-hybridized slide. After covered with a slide cover, the slide was incubated in HybChamber^{TM} with shaking overnight at 42°C.

2.3.7. Post-washing and scanning

The slide was carefully removed from the chamber and quickly placed in the staining dish, which contains 2×SSC and 0.2% SDS washing buffer. The cover slip was gently removed while the slide was in solution. The slide was transferred into 50 ml Falcon tube, which contained 2×SSC and 0.1% SDS washing buffer, and agitated vigorously for 10 min. The slide was washed in 1×SSC washing buffer with 0.1% SDS and finally at high-stringency in 0.1×SSC washing buffer with 0.1% SDS for 5min each. After a brief washing in ddH_2O, the arrays were dried by spinning for 2min at 400×g at room temperature and scanned using an AXON GenePix 4000 scanner that operating lasers at 633nm and 543nm to excite Cy5 and Cy3, respectively. Separate images were acquired for each fluorophore at a resolution of 10 µm per pixel.

2.3.8. Data collection and analysis

Data from each fluorescence channel were collected and stored as a separate 16-bit TIFF image. These images were analyzed to calculate the relative expression levels of each gene and to identify differentially expressed genes by using the GenePix Pro4.0 software. The analysis process could be divided into four steps.

First, the spots representing the arrayed genes must be identified and distinguished from spurious signals that can arise due to precipitated probe or other hybridization artifacts or

contaminants such as dust on the surface of the slide. This task is simplified to a certain extent because the robotic arraying systems used to construct the arrays produce a regular arrangement of the spotted DNA fragments.

The second step is the estimation of background. For microarrays, it is important the background be calculated locally for each spot, rather than globally for the entire image as uneven background can often arise during the hybridization process.

Third, following spot identification and local background determination, the background-subtracted hybridization intensities for each spot must be calculated. There are currently two schools of thought regarding the calculation of intensities - the use of the median or the mean intensity for each spot. As array analysis generally uses ratios of measured Cy3 to Cy5 intensities to identify differentially expressed genes, the mean intensity is easily fluctuated due to precipitated probe. Therefore, I think that the median intensity reflects the integrated intensities more objectively.

Fourth, following image processing, the data generated for the arrayed genes must be further analyzed before differentially expressed genes can be identified. The first step in this process is the normalization of the relative fluorescence intensities in each of the two scanned channels. Normalization is necessary to adjust for differences in labeling and detection efficiencies for the fluorescent labels and for differences in the quantity of starting RNA from the two samples examined in the assay. These problems can cause a shift in the average ratio of Cy5 to Cy3 and the intensities must be rescaled before an experiment can be properly analyzed. Further details can be found in results.

2.4. Northern blotting

DNA Microarray technology allows quickly analysis the differential expression of a good many of gene at one time. It has been extensively used in many research fields and proved to be an efficient tool. But we don't know how confident it is, so that the results from array must be verified by a reliable method. Northern blotting is a classical detection method for gene expression and is thought to be more dependable.

RNA for northern blotting is isolated as described in **section II 2.3.3.1.** The gelelectrophoresis, blotting, relabeled probes preparation, hybridization and detection were carried out using following protocols:

2.4.1 Agarose/formaldehyde gelelectrophoresis

A. Prepare gel

All vessels for northern blot were sprayed by Sigma RNaseZAP before using. 1.8 g agarose were dissolved in 130 ml water by heating with microwave oven and cooled to 60°C in a water bath. The flask was placed in ventilated case and added 15 ml of 10×MOPS buffer and 5 ml formaldehyde and well mixed by gentle shaking. Then the gel was poured and allowed to set. After solidification, the comb was removed and the gel was placed in the gel tank with sufficient 1×MOPS running buffer to cover the gel over ~ 1mm.

B. Prepare sample

The RNA sample (10µg) was adjusted to 10 µl with water, added 6 µl RNA denaturation mix (0.8 volume probe buffer), mixed by vortex, incubated 10 min at 65°C, and then cooled on ice for 2 min and microcentrifuged briefly to collect liquid.

C. Run gel

The RNA sample was loaded in gel and run in 1×MOPS running buffer at 100 volt for 120min.

2.4.2 Transfer of RNA from gel to membrane

A. The glass dish was filled with enough 20xSSC buffer.
B. Two pieces of Whatman 1MM paper were placed on the glass plate and wetted with 20xSSC buffer.
C. The gel was placed on the filter paper and squeezed out air bubble by rolling a glass pipet.
D. Four strips of plastic were wrapped and placed over the edges of the gel.
E. A piece of nylon membrane (Boehringer, Mannheim) was cut just large enough to cover the gel and placed on the surface of the gel. Air bubbles under the membrane were squeezed out.

F. The surface of the membrane was flooded with 20xSSC. Two sheets of Whatman 3MM paper with the same size as membrane were placed on top of the membrane.

G. Paper towels were putted on top of the Whatman 3MM paper to a height of ~15 cm, added a weight to hold everything in place, and then left overnight.

H. Preparation of membrane for hybridization: Paper towels and filter papers were removed. The membrane was recovered and flattened. The position of the wells on the membrane was marked in pencil to ensure that the up-down and back-front orientations were recognizable. Membrane was placed RNA-side-up in a in the UV-Stratalinker 2400 (Stratagene) to ultraviolet irradiation using Auto-Crosslinking-Program.

I. The membrane was baked in incubator at 70°C for 1h.

2.4.3. Preparation of DIG-labeling DNA probe

DIG-labeling DNA probes were synthesized by PCR using DIG probe synthesis kit. The kit contains all reagents required for the direct digoxigenin (DIG)-labeling of DNA fragments generated by PCR. The PCR amplification parameters (cycling conditions, template concentration, primer sequence and primer concentration) for each template and primer set in absence of DIG-dUTP were optimized before attempting incorporation of DIG. According to the length of the probe being labeled the concentration of DIG-dUTP has to be adapted: (1) < 1kb, use a 1:3 ratio of DIG-dUTP: dTTP (2) > 1kb, use a 1:6 ratio of DIG-dUTP: dTTP (3) > 3 kb, use a 1:6 ratio of DIG-dUTP: dTTP and substitute the expand long template enzyme mix for the expand High Fidelity enzyme mix included in the kit. The unlabeled positive control is always including in every experiment. It is required for evaluating probe-labeling efficiency. To check the success of the reaction, a portion of 5 µl of each reaction were electrophoresed on ethidum bromide (EtBr) containing gel. The presence of DIG in DNA make it run slower in the gel than unlabeled DNA, so the labeled probe should migrate slower the unlabeled control probe. The EtBr staining of the labeled DNA will be somewhat less than the unlabeled control DNA, because the polymerase is slowed by the presence of the DIG hapten.

2.4.4. Hybridization

A. Pre-hybridization

The membrane was placed RNA-side-up in a hybridization bag (Life Technologie). 10 ml Roche hybridization solution was added, and the bag was placed in the hybridization water bath and incubated by shaking at least 1 hr at 50°C.

B. Hybridization

Double-stranded probe was denatured by heating in PCR machine for 2 min at 95°C, and then transferred to ice. The desired volume of probe (usually 3 µl for 10 ml hybridization solution) was diluted in 1 ml hybridization buffer and pipetted into the hybridization bag, and then the incubation continued overnight at 50°C.

2.4.5. Washing, blocking and detection

The washing, blocking and detection buffers for the immunological detection of DIG-labeled probes are provided from DIG Wash and Block buffer set. Before beginning, the stock solutions were diluted into appropriate concentration.

A. The membrane was washed twice in a plastic container with 2× SSC buffer + 0.1% SDS for 5 minutes at room temperature.
B. The membrane was washed twice in 0.1× SSC buffer + 0.1% SDS for 15 minutes with stirring at 50°C.
C. The membrane was washed once with 100 ml Roche washing buffer for 2 min at room temperature.
D. The membrane was incubated in 50 ml Roche blocking solution (1×Maleic acid buffer and 1× Blocking solution) for 30 min at room temperature.
E. The membrane was incubated in 50 ml Roche blocking solution with 0.01% (v/v) DIG-antibody for 30 min at room temperature.
F. The membrane was washed twice with 100 ml Roche washing buffer for 15 min.
G. The membrane was equilibrated in 10 ml Roche detection buffer for 2-5 min.

H. The membrane was placed (with RNA side facing up) on a development folder (or hybridization bag) and applied approx. 4 drops of CSPD solution. The membrane was immediately covered with the second sheet of the folder to spread the substrate evenly and without air bubbles over the membrane, and then incubated for 10 min at 37°C.

I. The edges of the development folder were sealed around the damp membrane. The membrane was exposed to Roche Lumi-film for 10-60 min at 15-25° C and then developed.

2.4.6. Designing specific primers for AOX genes and 12-oxo-phytodienoic acid reductase (OPR3)

To distinguish between the four AOX genes in *Arabidopsis*, specific probes covering a region upstream the initiation codon and a region within the first exon of each gene were generated as described by Saisho (Saisho et al., 1997). The specific primer for OPR3 was designed within an exon region. For PCR-based probe synthesis, the following oligonucleotides were used:

AOX1a-fwd: CGTGTGAAGCGTATAAAGACGACAA,
AOX1a-rev: TCCTCCTTCATCGGAGTTTTCTC;
AOX1b-fwd: ATCCTAGAGTTCCGTAATTGCTA,
AOX1b-rev: TCTTCTCAAATGTCATCTTGCTG;
AOX1c-fwd: GAAGGCCTAATGAGCTAGTTGGA,
AOX1c-rev: CTTGCTGAAATCTCTCAGACCTC;
AOX2-fwd: AAGTCCGTATGAGATAACGGCAC,
AOX2-rev: CATCTTTGCTGTCTCTATTCCC.
OPR3-fwd: AAAACAGGTGGCGAGTTTTG;
OPR3-rev: CTCATCACTCCCTTGCCTTC.

2.5. Cellular respiration

Arabidopsis suspension cells were pretreated with 0.5 mM NOR3. At the different time points, cyanide-sensitive and cyanide-resistant (SHAM-sensitive) respirations were determined as oxygen uptake using an oxygen electrode. Suspension cells were placed in a Clark-type oxygen electrode cuvette at a density of 15-20 mg fresh weight ml $^{-1}$ in culture medium at 25°C. Inhibitors of cytochrome oxidase (1 mM KCN) and AOX (10 mM

salicylhydroxamic acid [SHAM]) were then added. Steady rates of respiratory O_2 uptake were determined after about 3 min.

2.6. Cell death assay

Cell death, measured as the loss of plasma membrane integrity, was detected by staining harvested suspension cells with Evans blue solution (0.025% [w/v] Evans blue in 0.1 mM $CaCl_2$, pH 5.6) for 10 min. Then stained cells were washed three times with 0.1 mM $CaCl_2$ (pH 5.6), and counted under a microscope. Counting was done for 4 independent experiments. A similar experiment to study cell as described for suspension cells was carried out in *Arabidopsis* plants. Because plants could absorb SHAM through roots and showed AOX inhibiting activity (Chivasa and Carr, 1998), 5 week old *Arabidopsis* plants were applied with AOX inhibitors (SHAM, 10 mM) through roots for 1 day, and then treated with 1250ppm gaseous NO for 10min. Pictures were taken 4 hours after NO treatment to indicate the cell death.

2.7. Determination of SA, ethylene and JA

Determination of SA and ethylene were done in work group headed by PD Dr. Christian Langebartels in BIOP. Extraction and quantization of free SA were performed by Wolfgang Mayr basically according to described by Meuwly (Meuwly and Metraux, 1993). SA was detected by using a Shimazu RF 535 fluorescence detector at excitation and emission wavelengths of 305 and 407 nm, respectively.

Ethylene accumulation and accumulation of free 1-aminocyclopropane-1-carboxylate (ACC) were measured by Rosina Ludwig with the use of a Perkin-Elmer Autosystem XL gas chromatograph equipped with a Porapak Q column (Supelco) and a flame ionization detector as described (Tuomainen et al., 1997).

Extraction and determination of JA was carried out by the Team of Dr. M. J. Mueller in University of Wuerzburg as described (Mueller et al., 1993).

2.8. NO detected by fluorescent microscopes

To analyze NO production by fluorescence microscopy, epidermal sections from the abaxial surface of leaves were peeled with a forceps and placed on a slide containing 30 µl of loading

buffer (10 mM Tris/KCl, pH 7.2) with DAF-2 DA at a final concentration of 10 µM (added from a 10 mM stock in DMSO). For cPTIO treatment, the epidermis pieces were rinsed in 100µM cPTIO for 1 min, before transferred to the slide containing DAF-2-DA. After overlaying the glass cover, the slides were placed under the microscope(s) and the images were taken within 10 min. For confocal laser scanning microscopy a Zeiss Axiovert 100M inverted microscope equipped with a confocal laser scanner was used in this study and sections were excited with the 488 line of an argon laser. Dye emissions were recorded using a 505-530 nm band pass filter, autofluorescence of chloroplasts was captured with a 585 nm long pass filter. Microscope, laser and photomultiplier settings were held constant during the course of an experiment in order to obtain comparable data. Images were processed and analyzed using the Zeiss LSM 510 software

Conventional fluorescence pictures were taken with a Zeiss Axioskop equipped with a standard FITC emission filter.

2.9. NO detection by electron paramagnetic resonance (EPR)

The rosette leaves were cut after treatment and frozen in liquid nitrogen at once. About 0.6g frozen leaves were crushed with a mortar and pestle under liquid nitrogen and incubated in a 1.2 ml buffered solution (50 mM N-2-hydroxyethyl-piper-az-ine-N'-2-ethane-sulphonate [HEPES]; 1 mM DTT and 1 mM $MgCl_2$, pH7.6) for 2 min . The mixture was centrifuged at 13,200g for 2min. The supernatant was added 300 µl of fresh made [Fe(II)(DETC)2] solution (2M $Na_2S_2O_4$, 3.3 mM DETC, 3.3 mM $FeSO_4$ and 33 mg/ ml BSA) (Tsuchiya et al., 1996), incubated for 2 min at room temperature and frozen again in liquid nitrogen. ESR measurements were performed on a Brucker ESP300 X-band spectrometer under following conditions: room temperature; microwave power 20mW; modulation amplitude 3G; scan rate ~2.5G/S; time constant, 164 msec.

III. Results

Currently, little is known about NO-responsive genes in *Arabidopsis*. To study the NO dependent gene induction, transcriptional analysis was carried out in *Arabidopsis* suspension cells by using a cDNA microarray that included about 330 defense-related genes encoding PR-proteins or proteins induced by oxidative stress, cold, UV, ozone, or heavy metals. Basically, the analytical methods are similar to published ones (Reymond et al., 2000; Wang et al., 2000).

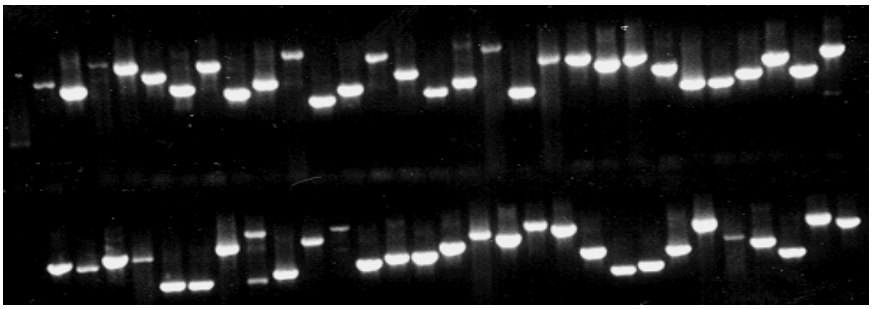
1. Preparation of the cDNA used for spotting

The cDNAs for microarray, which represent 330 stress genes (gene function, gene locus number and EST accession number see **Supplement Table S1**), were cloned in *E. coli* and cultivated in four different 96-well plates (signated with P0, D7, P1, and P2). The cDNA used for spotting were amplified by PCR using *E. coli* culture supernatant or isolated plasmid as template DNA.

200 µl PCR products of each gene are purified by 96 well multiscreen filter plates and dissolved in 50 µl of ddH_2O. 2 µl purified DNA were loaded on 1% agarose gel and electrophoresed. PCR bands are shown in **Figure 5**. The aims of electrophoresis are not only to detect the removing efficiency of unincorporated nucleotides and primers from the reaction products, but also to verify whether the PCR products from two parallel PCRs are identical. The success rate for single-band amplification is approximately 94.3% (313 genes); 5.7% of reactions (19 genes) yielded multiple bands or fail to amplify (notified with red color). Additional, there are obviously some weak bands on front of electrophoresis, which indicates that the purification is not completely and the unincorporated nucleotides and primers can not be fully removed by multiscreen filter plates.

(a)

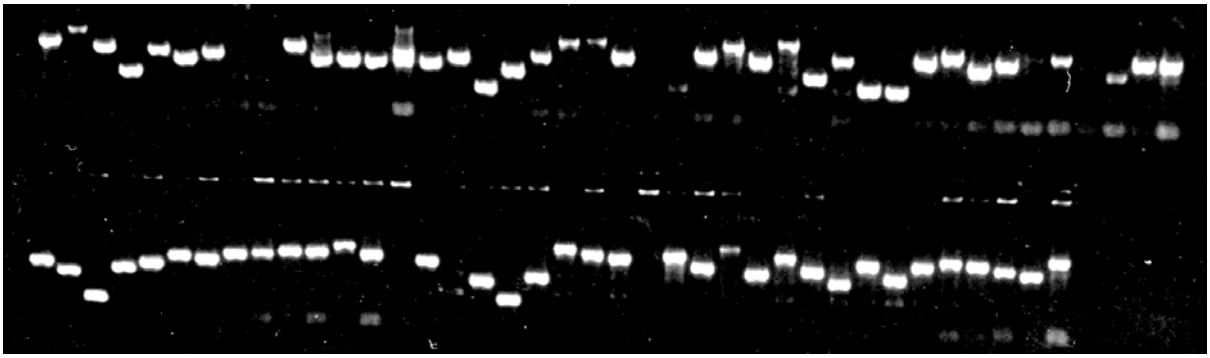
A1 A2 B1 B2 C1 C2 D1 D2 E1 E2 A3 A4 B3 B4 C3 C4 D3 D4 E3 E4 A5 A6 B5 B6 C5 C6 D5 D6 E5 E6



A7 A8 B7 B8 C7 C8 D7 D8 E7 E8 A9 A10 B9 B10 C9C10 D9 D10 E9 E10 A11 A12 B11 B12 C11 C12 D11 D12 E11 E12

(b)

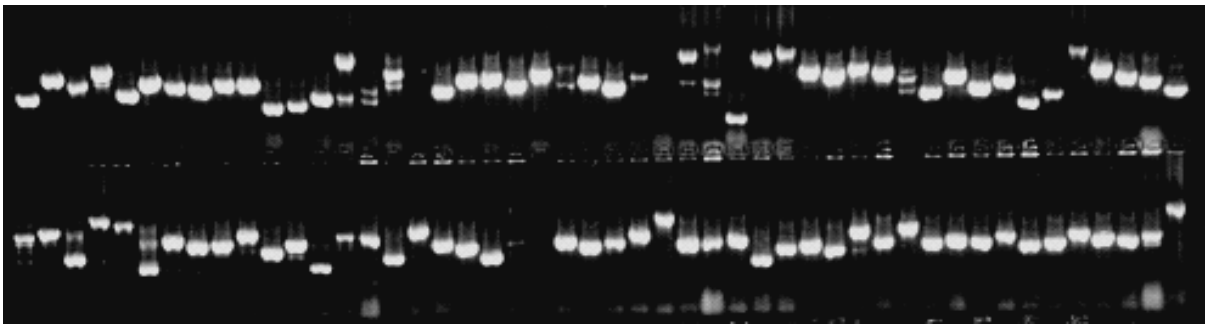
A1 A2 B1 B2 C1 C2 D1 D2 E1 E2 F1 F2 G1 G2 A3 A4 B3 B4 C3 C4 D3 D4 E3 E4 F3 F4 G3 G4 A5 A6 B5 B6 C5 C6 D5 D6 E5 E6 F5 F6 G5 G6



A7 A8 B7 B8 C7 C8 D7 D8 E7 E8 F7 F8 G7 G8 A9 A10 B9B10 C9C10 D9D10 E9 E10 F9F10A11A12 B11B12 C11C12 D11D12 E11E12 F11F12

(c)

A1 A2 B1 B2 C1 C2 D1 D2 E1 E2 F1 F2 G1 G2 H1 H2 A3 A4 B3 B4 C3 C4 D3 D4 E3 E4 F3 F4 G3 G4 H3 H4 A5 A6 B5 B6 C5 C6 D5 D6 E5 E6 F5 F6 G5 G6 H5 H6



A7 A8 B7 B8 C7 C8 D7 D8 E7 E8 F7 F8 G7 G8 H7 H8 A9 A10 B9 B10 C9 C10 D9D10 E9E10 F9F10 G9 G10 H9 H10 A11A12 B11B12 C11C12 E11E12 F11F12 G11G12 H11 H12

(d)

Figure 5: Electrophoresis of PCR products of stress genes on 1% agarose gel. (a) Plate P0; (b) Plate D7; (c) Plate P1; and (d) Plate P2. The labels indicate the position of cDNA clones on the 96-well microtiter plates (Gene locus number and EST Accession number can be found in supplement **Table S1**).

2. Microarray analysis of transcripts of suspension cells after NO treatment

NO-donors such as nitrosothiols or NOR-3 ((E)-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexene-amide) are excellent tools to investigate NO-signaling both in the animal and the plant field, especially for treatment of cell layers and/or cell suspensions. In animal systems, NOR-3 has been proven to generate only biologically inactive by-products (Kita et al., 1994), so NOR-3 was chosen as NO donor in the NO burst simulation experiments.

After NOR3 treatment, cells were filtered at 4 time points up to 24h, and total RNA was purified. The cDNA made from each sample was labeled with the fluorescent dye Cy5 and mixed with a reference probe consisting of cDNA made from mRNA from untreated cells and labeled with a second fluorescent dye Cy3. The two populations of labeled cDNAs were simultaneously hybridized with the cDNA microarray. After scanning, data from each fluorescence channel were collected and stored as a separate 16-bit TIFF image. These images were analyzed to calculate the relative expression levels of each gene and to identify differentially expressed genes by using the GenePix Pro4.0 software.

2.1 Data analysis and normalization

Data analysis was done as described in Section **Method 2.3.7**. Additionally, I have to supplement some details to the data normalization. The essential importance of normalization for microarray has been fully discussed (Chen et al., 2003; Edwards, 2003; Wilson et al., 2003). The most favorite strategy of normalization is using some subset of the genes such as housekeeping genes that are thought to be less regulated by the treatment in experiment. The intensity and average ratio (Cy5/Cy3) of the analyzed genes are adjusted and rescaled based on the calculation of fluorescent intensity and ratio of control genes. But NO is a multiple signal molecule, which regulates diverse physiological processes. Moreover, few genes activated by NO are known. It is difficult to designate the control genes for NO treatment. Another simple strategy is uses total measured fluorescence intensity. The assumption underlying this approach is that the total mass of RNA labeled with either Cy3 or Cy5 is equal. While the intensity for any one spot may be higher in one channel than the other, when averaged over thousands of spots in the array, these fluctuations should average out. Consequently, the total integrated intensity across all the spots in the array should be equal for both channels.

Additionally, care must be taken in handling genes expressed at low levels. Statistical fluctuation in the measured levels can cause a significant variation in the ratios that are calculated and inefficiencies in labeling for either of the two dyes can cause these low intensity genes to disappear from the arrays. Typically, I only use spots in the final analysis where the intensities in both channels are two standard deviations above background.

Following normalization, data are typically analyzed to identify genes that are differentially expressed. Most published studies have used a post-normalization cutoff of two-fold up- or down-regulation to define differential expression (Chen et al., 2002). But I used a stricter standard: induction or repression of a gene was defined by a minimum 2.5 fold change in its transcript level. In order to separate genes that are truly differentially expressed from stochastic changes, I typically carried out three independent microarray assays starting from independent mRNA isolations and define differential expression based on their consensus.

2.2. Dynamics of gaseous NO-inducible gene expression

After analysis and normalization, an overall view of the gene expression data can be presented by scatter plots, from which the transcript changes are indicated (**Figure 6**). The scatter plots show that 2h after NO treatment, many genes are both induced and repressed greater than 2-fold. 24h after NO treatment, many gene inductions return to the normal level, which can be indicated by fewer points remaining outside the guide lines.

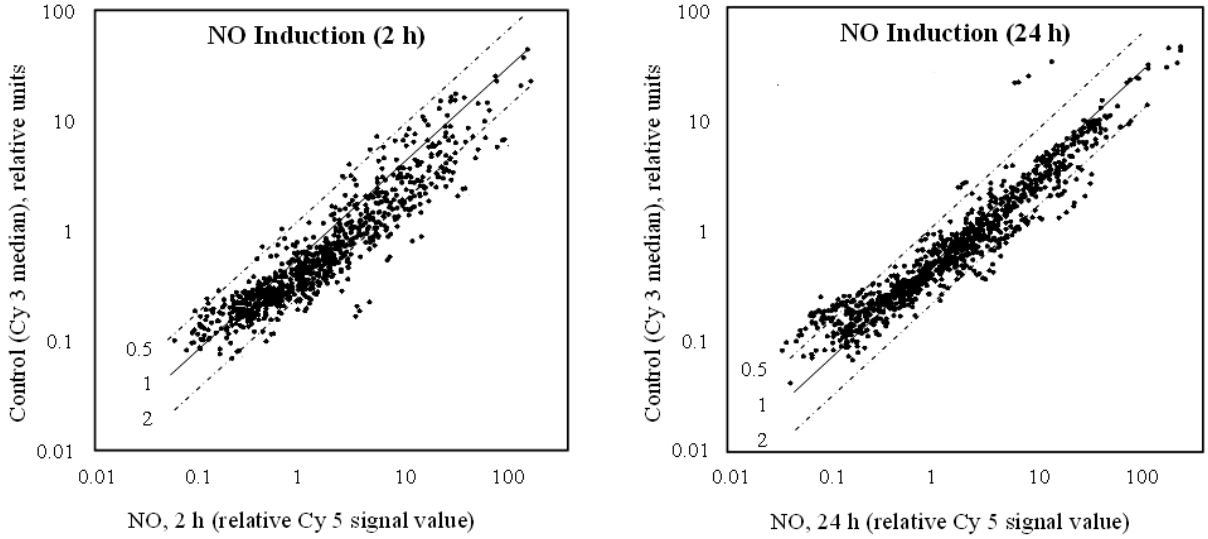


Figure 6: Microarray scatter plot of 2h and 24h after NO treatment in suspension cells. Signal intensities from control samples (*y*-axis) were plotted over NO-treated samples (*x*-axis) for 2h (left) and 24h (right). Guide lines are provided in the plots showing when signal ratios are 2 and 0.5 (–2).

To identify the genes with differently expression, more rigorous standard has been used (see **Section 2.1**). These strict criteria implicate that the procedure ignores such genes with relatively low basal expression ratios, even if those genes showed high induction by NO. To obtain specific gene expression, the NO-treatment was adjusted to yield only moderate changes in transcriptional activity. At the conditions applied (0.5 mM NOR-3) the cells did not show any symptoms such as cell death, and only about 10% of all transcripts showed transient accumulation with highest expression around 2h and 4h post treatment, and rapid downregulation after 24h (**Table 2**). Array data were based on four independent replicates and the standard deviation (SD) were shown on the right side of each ratio. Additional results of DNA array (complete data) can be found in supplement **Table S2**.

Table 2. DNA array analyses of transcripts in *Arabidopsis* cell suspension.

Function	Gene locus	2h	SD	4h	SD	8h	SD	24h	SD
Alternative oxidase 1a precursor (AOX1a)	At3g22370	8,4	0,6	2,8	0,2	2,5	0,4	1,7	0,2
Glutathione s-transferase (GST)	At2g29420	5,9	1,2	4,5	0,3	4,2	0,1	2,3	0,2
5'-Adenylylphosphosulfate reductase (PRH43)	At1g62180	5,0	0,4	2,0	0,1	1,6	0,1	1,4	0,2
Mac9 unknown protein	At5g61820	4,4	0,4	5,1	0,5	5,2	0,2	3,1	0,4
Heat shock cognate 70kD protein	At3g09440	4,3	0,2	3,7	0,2	3,0	0,1	1,1	0,0
Cytoplasmic isozyme	At2g36460	4,2	0,3	2,5	0,1	2,0	0,2	2,0	0,2
Glutathione-conjugate transporter	At2g34660	4,2	0,3	2,6	0,1	2,8	0,1	1,8	0,4
Glutathione s-transferase (GST1)	At1g02930	4,0	0,3	4,5	0,2	3,2	0,2	1,1	0,1
Peroxidase (POX)	At3g49120	3,8	0,6	2,4	0,3	1,9	0,2	0,8	0,6
Isoflavonoid reductase homologue	At1g75280	3,7	0,5	2,6	0,3	1,9	0,1	1,5	0,2
Glutathione peroxidase (ATGP1)	At4g11600	3,6	0,1	4,2	0,3	4,3	0,2	1,7	0,1
Glutathione S-transferase (GST11)	At1g02920	3,6	0,3	4,5	0,3	1,9	0,2	0,6	0,0
Serine/threonine kinase- like protein	At4g23220	3,4	0,2	2,5	0,1	2,0	0,1	2,1	0,2
Allene oxide synthase (AOS)	At5g42650	3,3	0,7	1,5	0,2	1,8	0,3	1,5	0,5
Ascorbate peroxidase (APX1)	At1g07890	3,3	0,2	2,3	0,3	2,0	0,2	1,4	0,1
Pre-hevein-like protein (similar to PR 4)	At3g04720	3,1	0,1	3,5	0,2	1,4	0,1	1,2	0,1
pEARLI1	At4g12480	3,0	0,5	1,1	0,2	1,3	0,2	3,9	0,7
Beta-1,3-endoglucanase (BG4)	At3g55430	3,0	0,2	2,5	0,1	1,9	0,2	1,7	0,2
PRH19 5'-adenylylsulfate reductase	At4g04610	3,0	0,6	1,5	0,1	1,3	0,1	1,4	0,2
Seed imbibition protein	At5g20250	2,8	0,2	1,7	0,1	1,7	0,2	1,9	0,2
Peroxidase like protein	At2g38380	2,7	0,2	2,1	0,0	3,8	0,4	10,1	1,0
AP2 domain containing protein RAP2.5	At3g15210	2,6	0,3	1,6	0,2	1,6	0,1	1,9	0,1
Auxin-responsive protein IAA10	At1g04100	2,5	0,4	1,2	0,1	1,3	0,1	1,7	0,1
Alpha DOX1 fatty acid dioxygenase	At3g01420	2,4	0,3	3,5	0,4	2,2	0,2	4,6	0,7
Universal stress protein	At3g53990	2,3	0,2	3,5	0,4	3,1	0,2	1,4	0,0
Blue copper binding protein	At5g20230	2,3	0,2	2,5	0,1	1,3	0,4	0,6	0,0
Catalase (Cat1)	At1g20630	2,3	0,1	2,2	0,1	2,7	0,2	2,0	0,5
Beta - glucanase 3 (BG3)	At3g57240	2,2	0,2	1,2	0,1	1,5	0,1	2,9	0,7
Polyubiquitin (ubq3)	At5g03240	2,2	0,1	2,6	0,1	2,2	0,1	1,9	0,1
Glutaredoxin-like protein (APR4)	At5g40370	2,2	0,1	2,0	0,2	2,8	0,1	2,2	0,3
Glutathione peroxidase 2 (GPX2)	At2g31570	2,1	0,2	2,7	0,3	2,8	0,3	2,2	0,1
Flavanone-3-hydroxylase, F3H	At3g51240	2,1	0,2	1,8	0,1	1,1	0,0	3,0	0,4
Chalcone flavanone isomerase	At3g55120	2,1	0,2	1,2	0,0	1,4	0,1	2,6	0,3
Metallothionein (AtMt1)	At1g07610	1,9	0,1	3,3	0,1	4,2	0,3	3,6	0,5
Cytochrome P450 (CYP83B1)	At4g31500	0,7	0,1	0,5	0,1	0,5	0,0	0,7	0,0

The genes with weak signals (lower than 2 fold local background) were neglected by grey scripts. The genes with induction over 3.5 fold are marked by red colored boxes, the ratio below 0.5 are marked with green boxes, and with induction between 2.5-3.5 fold by orange colored boxes. Array data were based on four independent replicates and the standard deviation (SD) were shown on the right side of each ratio.

DNA microarrays proved itself a high throughput vehicle for the exploration of gene expression profile. Among the most interesting genes are defense genes (e.g. beta-1, 3-endoglucanase, beta glucanase, pre-hevein-like protein, universal stress protein), an array of anti-oxidant genes (e.g. alternative oxidase, ascorbate peroxidase, glutathione peroxidases, neutral peroxidase, catalase and glutathione S-transferases), transcription factors (e.g. AP2 domain containing protein RAP2.5, AtERF4), genes related to metal metabolism (e.g. blue copper binding protein; Metallothionein AtMt1), and many inductions of unknown genes (**Table 2**). Additionally, two important genes (allene oxide synthase and alpha dioxygenase peroxidase), which are reactive intermediates in the oxylipin pathsways of fatty acid oxygenation in plants(Saffert et al., 2000; Schaller, 2001), are surprisingly found to be induced by NO treatment. The induction of AOX1a is further investigated due to its specific role in plants in following section.

3. Identification of the alternative oxidase (AOX) as NO counteracting principle

Among the most interesting genes induced by NO was alternative oxidase (AOX) (**Table 2**). Plants possess two terminal oxidases operating side by side in the electron transport chain. One is cytochrome c oxidase (COX); the other one is termed as alternative oxidase (AOX). AOX catalyzes the oxidation of ubiquinol and the reduction of oxygen to water, bypassing the final steps of the cytochrome c pathway. Unlike the cytochrome c pathway, which is coupled to proton translocation, electron transport from ubiquinol to AOX is nonphosphorylating and releases energy as heat. The two pathways can be differentiated by inhibitors such as cyanide or SHAM (acting at AOX). AOX was long recognized as a cyanide-resistant component of oxygen consumption and was a dominant component of respiration in thermogenic inflorescenes (Meeuse, 1975). NO has been found to inhibit cellular respiration through its action at COX. Strikingly, unlike cytochrome c oxidase, AOX is barely affected by NO. Therefore, it has been suggested that AOX plays a role in NO tolerance of higher plants (Millar and Day, 1997). The inhibitory effect of NO on respiration is believed to its most deleterious side effect, not only because of reduced respiration, but mainly because of generation of $O_2^{\cdot -}$ due to a dramatically reduced ubiquinone pool (Shiva et al., 2001).
DNA microarrays indicate that NO treatment of *Arabidopsis* plants or cell cultures induces AOX transcription. Here I provide further evidence that AOX plays a role in NO tolerance of *Arabidopsis*.

3.1. Northern blot verify the AOX induction by NO

An examination of transcript level is obviously an indirect means to examine AOX abundance, but it is a sensitive measure of how AOX gene expression may be changing in response to an experimental treatment. To verify the array data on *AOX1a* expression, cells were treated with NOR-3 as described (**II 2.2**). Total RNA was extracted at the indicated time-points and subjected to Northern blot hybridization (**Figure 7**). The strong induction of AOX1a mRNA in response to NO confirmed the expression data obtained by the cDNA array (**Table 2**). The induction intensity was dependent on NO dosage. Treatment with 0.1 mM NOR3 only slightly induces AOX1a. 0.5 mM NOR3 strongly induced AOX1a 30min after treatment. One hour after treatment transcription was peaking, followed by a slow decrease.

At the final time point of experiment (24h after treatment), AOX1a still shows slight induction, as appears in 0.1 mM NOR3 treatment.

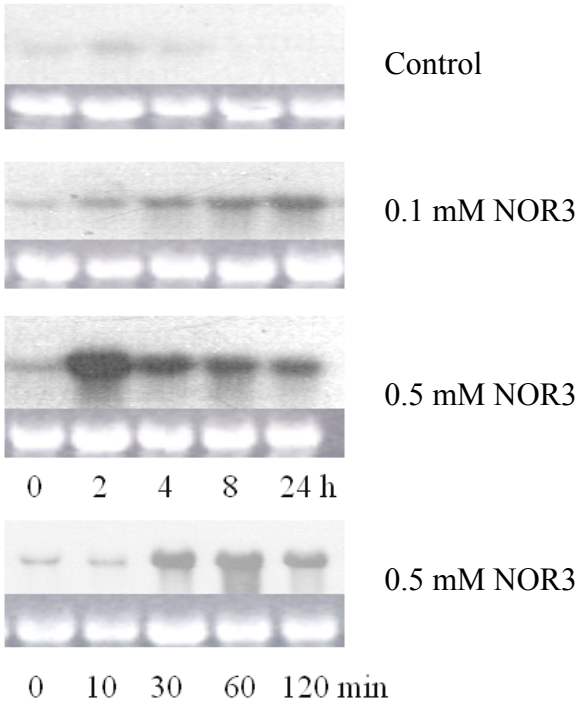


Figure 7: *AOX1a* gene expression in *Arabidopsis* suspension cells by NOR3, a potent NO donor. Cell suspension cultures were treated with the NO donor NOR3 (0.1 and 0.5 mM, respectively). Cells were collected at the times indicated for RNA preparation (0-24 h). The lower panel shows the time course of AOX1a induction during the first 2 h. Ethidium bromide staining shows loading of the gels.

To verify that *AOX1a* induction is indeed a result of NO treatment, the cell permeable NO scavenger carboxy-2-phenyl-4,4,5,5-tetramethylimidazolinone-3-oxide-1-oxyl (cPTIO) was applied. cPTIO is highly specific for NO scavenging, does not react with any ROS (Barchowsky et al., 1999) and has been used to (transiently) block NO-dependent cell death and defense gene activation in tobacco and soybean (Delledonne et al., 2001; Durner et al., 1998). 250 µM cPTIO led to strong suppression of AOX1a induction by NO (**Figure 8**).

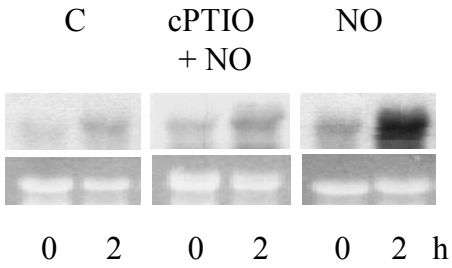


Figure 8: cPTIO suppresses NO-induced AOX gene expression in *Arabidopsis* suspension cells. Cell suspension cultures were treated with NOR-3 (0.5 mM) in presence or in absence of the NO-scavenger cPTIO (0.5 mM). Cells were harvested before addition of the chemicals or at the time-point with highest *AOX1a* induction (2h). Northern Blots were probed with cDNAs for *AOX1a.* Shown is the region between 1.8 and 1.0 kb. Ethidium bromide staining shows loading of the gels.

3.2. Identification of other AOX homolog in *Arabidopsis*

In *Arabidopsis*, AOX describes a small enzyme family consisting of 4 members. Identities between the deduced amino acid sequences of the four AOX proteins are: AOX1a/AOX1b, 77%; AOX1a/AOX1c, 73%; AOX1a/AOX2, 70%; AOX1b/AOX1c, 81%; AOX1b/AOX2, 68%; AOX1c/AOX2, 67% (Saisho et al., 1997; Tsuji et al., 2000). Since cDNA arrays like ours are highly sensitive, but also less specific compared with oligonucleotide based arrays, we monitored transcript accumulation by Northern analysis using highly specific probes. Saisho et al. showed that the 5'-untranslated regions, which were not highly conserved amongst the different gene family members, could be used as copy-specific probes for standard Northern analysis (Saisho et al., 1997). In order to identify the AOX genes in our cell suspension cells, PCR was performed using gene-specific primers that correspond to the region upstream of the putative initiation codon and the region downstream of the putative termination codon of *AOX1*a, *AOX1*b, *AOX1c* and *AOX2* (Saisho et al., 1997) (**Figure 9**). The probes constructed for Northern hybridization were based on the forward primers used in **Figure 10** (AOX1a-fwd, AOX1b-fwd, AOX1c-fwd, AOX2-fwd) in combination with reverse primers targeted to the coding regions within the first exon of each AOX gene (Primer sequences are given in **II 2.4.6**). These primer sequences have been originally described by Saisho *et al.* (Saisho et al., 1997).

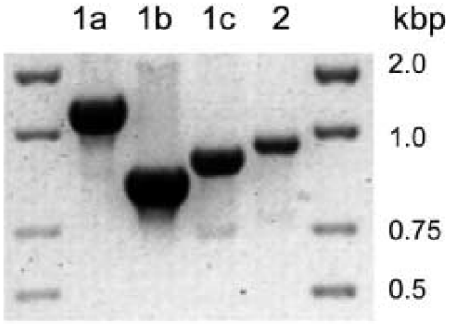


Figure 9: Electrophoretic gel of PCR-amplified AOX fragments from genomic DNA extracted from *Arabidopsis* cell suspensions. The gene-specific primers for the individual AOX genes of *Arabidopsis* were chosen as suggested by Saisho (Saisho et al., 1997). The amplified AOX fragments were separeated on a 1% agarose gel. Numbers on the right indicate sizes of fragments in kbp.

Under the conditions used, only the transcript encoding AOX1a could be detected. The strong induction of AOX1a by NO confirmed the expression data obtained by the cDNA array (**Table 2**) and is also identical to the results using cDNA as probe (**Figure 9**). In case of AOX1b a weak signal, albeit not induced by NO, could be detected (**Figure 10**). It should be noted, however, that the blot strips with AOX1b, AOX1c and AOX2 were exposed much longer than that containing AOX1a.

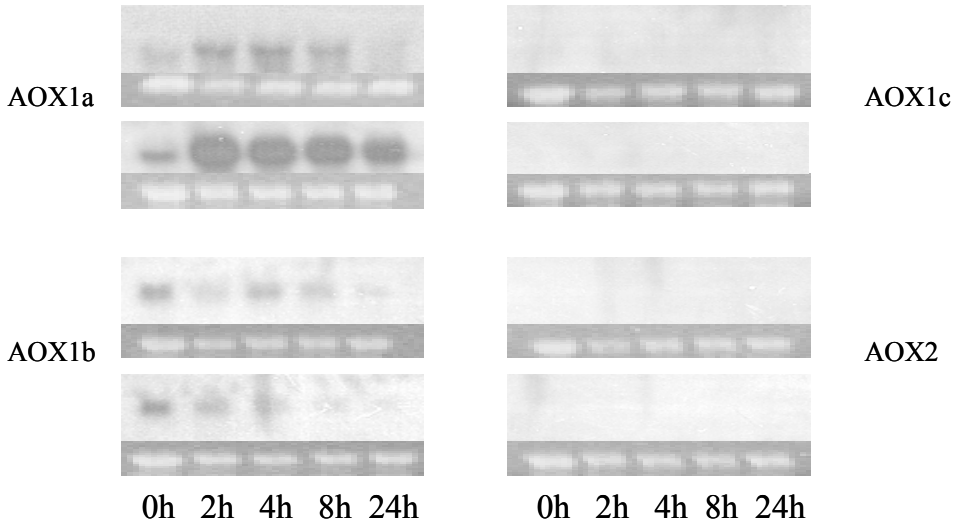


Figure 10: AOX gene expression in *Arabidopsis* suspension cells by NOR-3. Cell suspension cultures were treated with the NO donor NOR-3 (0.5 mM). Cells were collected at the times indicated for RNA preparation (0-24 h). Northern blots were probed with cDNAs for AOX1a, AOX1b, AOX1c and AOX2.

3.3. Identification of the transcript of AOX1a in mutants

Noteworthy, the fast induction by NOR-3 with full transcriptional activation within the first 2 hours precedes the generation of reported downstream messengers of NO such as SA or cGMP. To dissect the signal requirements for the expression of AOX, *Arabidopsis* mutants and/or transgenes with impaired signaling pathways were analyzed. I was predominantly interested in SA signal transduction mutants or transgenes. However, when treated with gaseous NO neither the SA signaling mutations *pad4* and *npr1*, nor the presence of NahG gene (mutants and/or transgenes are reviewed by (Glazebrook, 2001)) did influence the NO induction of AOX as seen in the wildtype (**Figure 11**). These data suggest that NO induces *AOX* independently of SA. Other mutation (such as etr1 and dnd1) also didn't inhibit the AOX induction. Generally, the AOX induction intensity in plants treated with gaseous NO is evidently weaker than in cell suspension treated with NOR3.

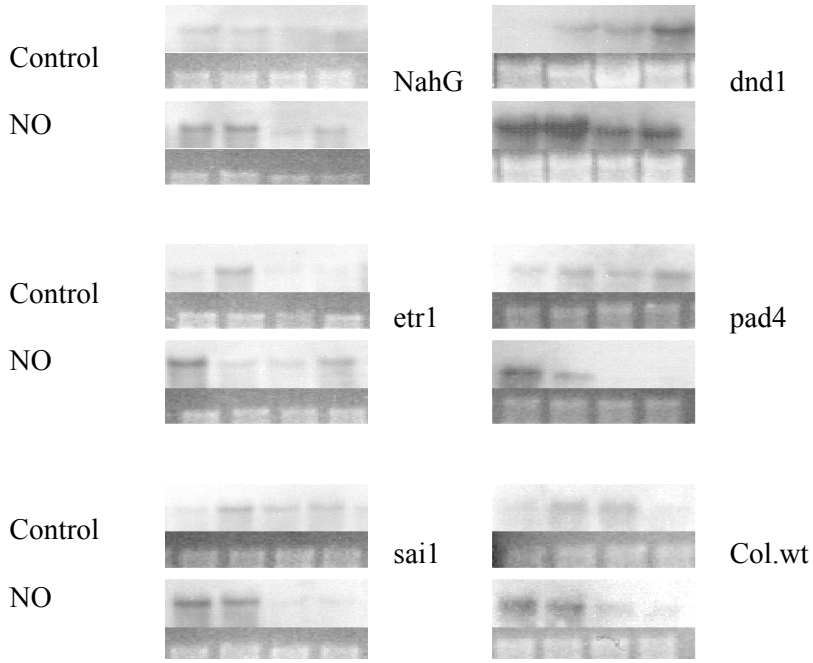


Figure 11: *AOX1a* gene expression in *Arabidopsis* mutants in response to NO treatment. Total RNA was isolated from leafs after incubation with NO (10min at 1250 ppm gaseous NO) and subjected to northern analysis. Shown are two different ecotypes (Columbia and Nossen), as well as the following mutants: *dnd1*, defense, no death; *pad4*, phytoalexin-deficient; etr1, ethylene resistant; *sai1*, salicylic acid insensitive. Mutants and NahG plants all have Columbia background except *sai1* (Nossen). Ethidium bromide staining shows loading of the gels.

3.4. Determination of the respiration after NO treatment

Now a question is open, whether the reported inhibition of cytochrome c respiration (Brunori et al., 1999) and induction of AOX by NO as shown by **Figure 10** and **Table 2** could be verified by measuring respiratory oxygen uptake? The effect of NO on cellular respiration is summarized by **Figure 12**. *Arabidopsis* cells were pretreated with 0.5 mM NOR-3. At the indicated time points, cyanide-sensitive (cytochrome c pathway) and cyanide-resistant (SHAM-sensitive AOX pathway) respiration were determined as oxygen uptake using an oxygen electrode. As shown by **Figure 12**, the decline of cytochrome c dependent oxygen uptake was paralleled by an increase of SHAM-sensitive respiration. I did not attempt to analyze regulation, substrate availability or other biochemical and physiological parameters. However, these data suggest that NO inhibited cytochrome c respiration and induced AOX respiration.

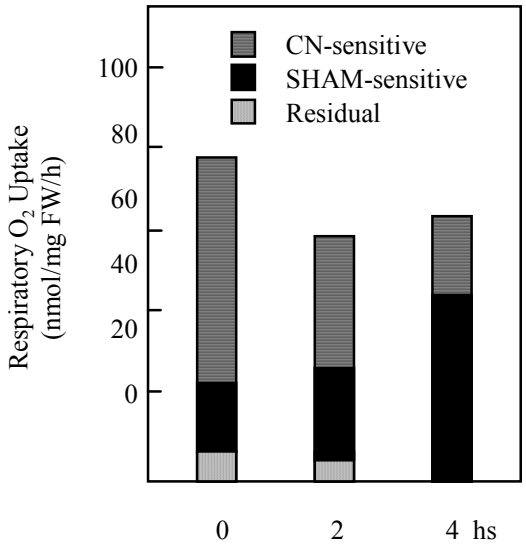


Figure 12: Respiratory characteristics of *Arabidopsis* suspension cells treated with NOR3. Cell cultures were treated with 0.5 mM NOR3. At the indicated time points, cyanide-sensitive and cyanide-resistant (SHAM-sensitive) respiration was determined as oxygen uptake using an oxygen electrode. KCN was 1 mM, SHAM 10 mM. Respiratory O_2 uptake resistant to both KCN and SHAM is expressed as residual respiration. Data represent mean values of three independent experiments.

3.5. Identify the role of AOX in protection the cell from NO-induced cell death

Mitochondrial AOX has been proposed to dampen the generation of reactive oxygen species, namely superoxide ($O_2\cdot^-$), during periods of high respiration, and during inhibition of the cytochrome c oxidase by inhibitors such as cyanide or NO (Millar and Day, 1997; Yip and Vanlerberghe, 2001). Since the combined action of $O_2\cdot^-$ and NO can result in highly deleterious oxidative and nitrosative damage, we analyzed the effects of NO and SHAM on cell death. Cell death, measured as the loss of plasma membrane integrity, was detected by staining harvested suspension cells with Evans blue. As shown in **Figure 13**, treatment with NO alone (applied as 0.5 mM NOR-3) yielded a moderate degree of cell death only after prolonged incubation (24 h). In contrast, the simultaneous presence of SHAM (2 mM) dramatically increased cell death. After 6 h, more than 80% of all cells were dead. Treatment with SHAM alone did not affect the cells.

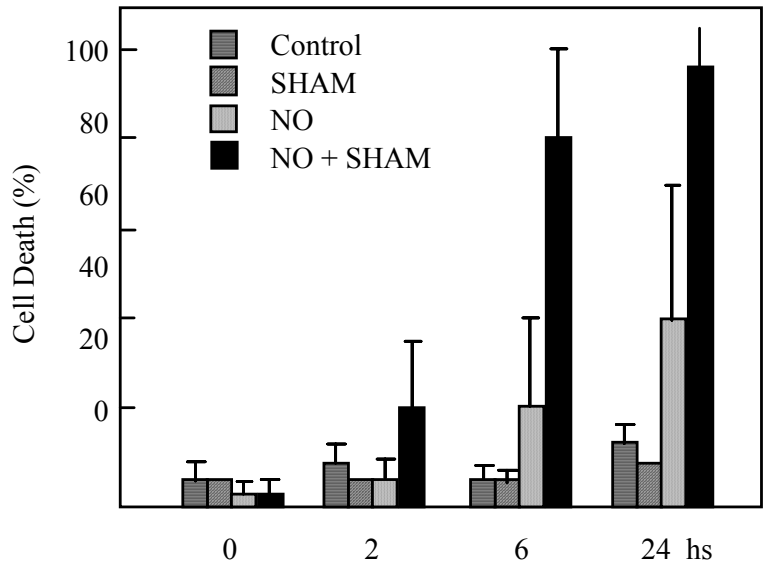


Figure 13: AOX protects *Arabidopsis* suspension cells from NO-induced cell death. Cells were treated with NOR3 (0.5 mM) and/or SHAM (10 mM). At the indicated time points, cell death was estimated by Evan's blue staining. Data are the averages from four independent experiments.

While Evans blue is a widely used cell death assay, cells at a late stage of dying are frequently not stained. Therefore we also performed a counterstain with fluoresceindiacetate, staining live cells only. The results (on a dead cell basis) were highly similar as presented in **Figure 13**. To visualize cell death, *Arabidopsis* suspension cells were analyzed with a confocal laser

scanning microscope (**Figure 14**). The picture was taken 4 h after treatment with NO/SHAM. Untreated cells show high fluorescence (green), while treatment with NOR-3 (0.5 mM) and SHAM (2-10 mM) almost completely abolished the fluoresceindiacetate signal.

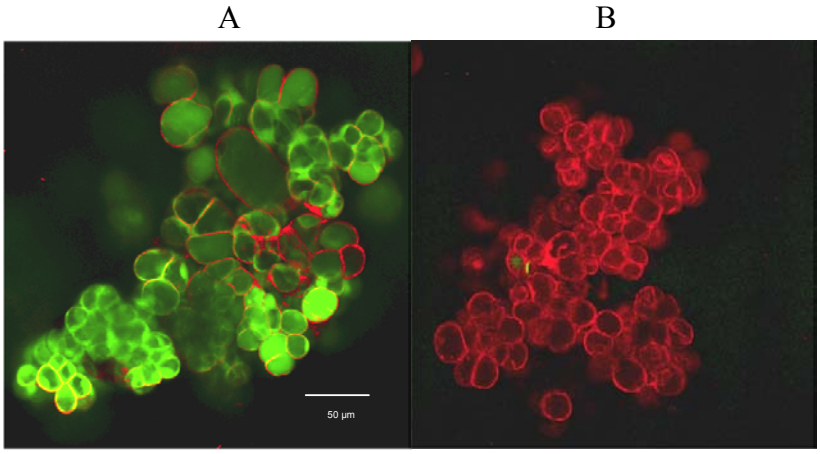


Figure 14: Visualization of SHAM/NO-induced cell death in *Arabidopsis* cells. *Arabidopsis* suspension cells were observed with a confocal laser scanning microscope. Cells were not treated (A) or treated with NOR3 (0.5 mM) and SHAM (10 mM) for 4h to induce cell death (B). Staining was with fluoresceindiacetate and Evans blue.

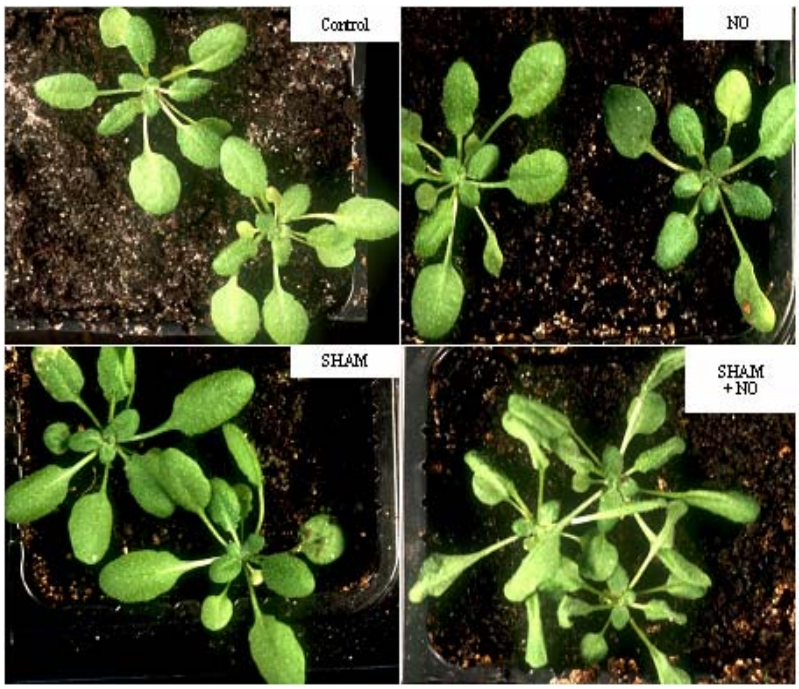


Figure 15: AOX protects *Arabidopsis* plants from NO-induced damage. Plants were watered with or without SHAM (1 mM) for 24 h and treated with gaseous NO (when indicated) for 10 min. Pictures were taken 2 h after NO-treatment.

SHAM could be absorbed by plants through the roots and showed its inhibition activity to AOX (Chivasa and Carr, 1998). We watered the *Arabidopsis* plants with (or not with) 1 mM SHAM solution for 1 day as described by Chivasa and Carr (1998). Control plants, or plants treated only with NO or SHAM did not show any symptoms or any visible injury even after 2 days. But treatment with NO and SHAM together dramatically induced strong cell death (**Figure15**). The pictures were taken 1h after treatment of gaseous NO. Overall, these results (**Figures 13, 14,** and **15**) suggest that AOX might fulfill an important function for cellular homeostasis under NO stress.

4. Microarray transcripts analysis of *Arabidopsis* plants after gaseous NO treatment

To reveal the NO-dependent gene induction in whole plants, a NO burst simulation was carried out in controlled environment cabinets with complete instrumentation to adjust and control gaseous NO through an electrochemical sensor. *Arabidopsis* plants were treated with NO concentrations of 1250 ppm for 10 min. Gaseous NO was likely to access into plant leaves through stomata as described (Wellburn, 1990). At the conditions applied the plants did not show any symptoms of senescence or lesions (cell death, data no shown). Array processing and analysis of our bouquet array were as described in the section of transcripts analysis in cell suspension.

4.1. Dynamics of gaseous NO-inducible gene expression

The temporal program of transcription was studied in gaseous NO treated *Arabidopsis* leaves. At 4 time points up to 24 hr after NO treatment, leaves were harvested and mRNA was purified. To avoid the interference of circadian rhythm, leaves of untreated plants were also harvested as control simultaneously at each time point. The cDNA made from each sample was labeled with the fluorescent dye Cy5 and mixed with a reference probe consisting of cDNA made from mRNA from untreated plants and labeled with a second fluorescent dye Cy3. The two populations of labeled cDNAs were simultaneously hybridized with the cDNA microarray; after scanning, the signal intensity for each gene was integrated. The samples of the pseudocolor images of the results obtained for each time points (1h, 3h, 8h, and 24h after NO treatment) are shown in **Figure 16**. Here, marked changes in transcript levels relative to those in the control plants are visible. Genes induced or repressed after NO treatment are represented as red or green signals, respectively. Genes expressed at approximately equal levels between treatments appear as yellow spots. The intensity of each spot corresponds to the absolute amount of expression of each gene. About 10% of all genes showed change in transcription between 1 and 3h post treatment, and rapid down regulation after 8h. At the last two time points (8h and 24h), there are almost no gene expression differences (**Figure 16**).

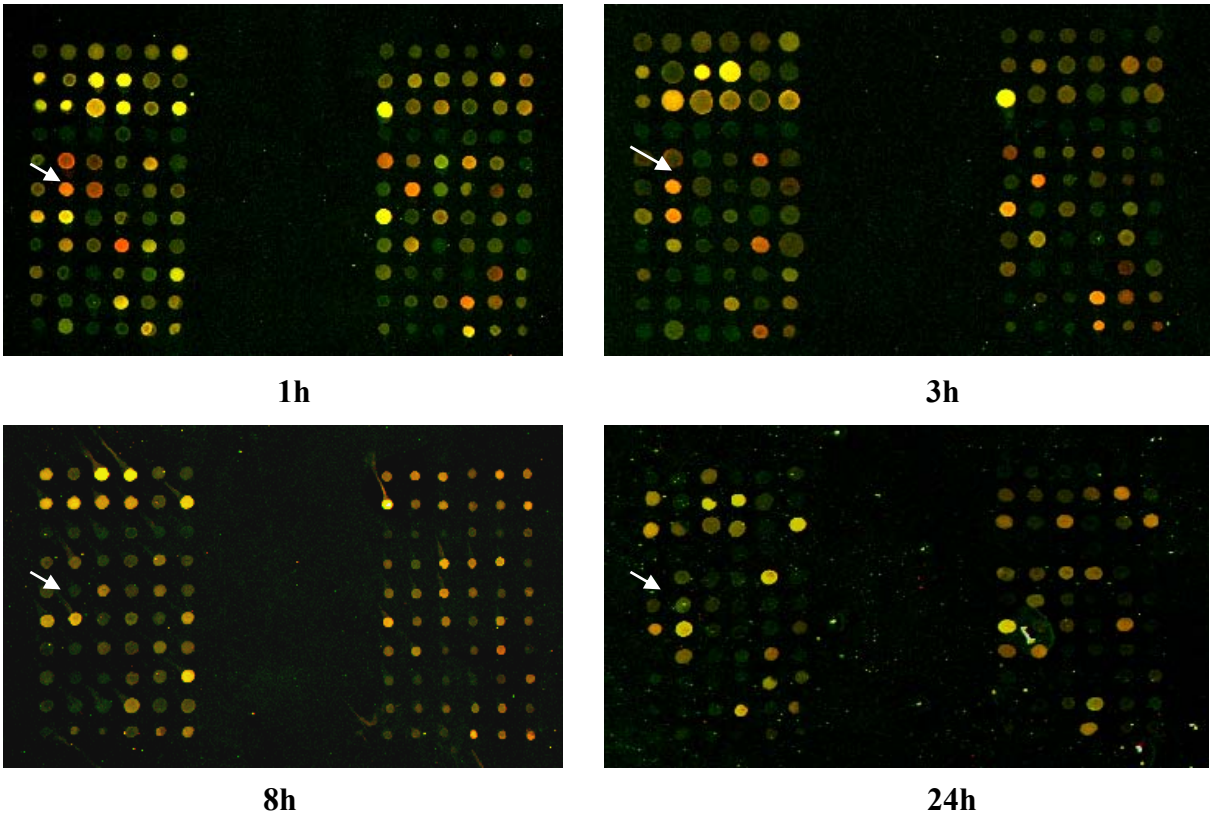


Figure 16: Typical samples of cDNA microarray analysis of gene expression after gaseous NO treatment. A fluorescence labeled cDNA probe was prepared from mRNA isolated from control *Arabidopsis* leaves by reverse transcription in the presence of Cy3-dUTP. A second probe, labeled with Cy5-dUTP, was prepared from leaves that were gaseous NO treated (1h, 3h, 8h, 24h). Arrows indicate the expression of the same gene in different time points.

Although cDNA arrays are highly sensitive and produce robust data, the reliability of the arrays data is prevalently concerned (Finkelstei et al., 2002). To ensure the confidence of the arrays results, I verified the transcript accumulation by Northern analysis. The probes constructed for Northern hybridization were based on the primers used to generate the cDNA for the array. Six selected NO-induced genes (calmodulin 3 [CaM 3, At3g56800], AP2 domain containing protein RAP2.5 [AtERF4, At3g15210], dehydration-induced protein [ERD15, At2g41430], blue copper-binding protein [At5g20230], glutathione S-transferase [GST, At2g29420], and endo-1, 4-beta-D-glucanasse [At4g30280]) were monitored by northern blots (**Figure 17**). The northern blots show almost identical results to that from microarray (**Table S3**). These results reinforce the cognition that the NO treatment in my experiment (1250ppm for 10 min) induced quick, but only transient transcript accumulation.

In the following section, only results of 1h and 3h after NO treatment are shown, although all the time points have been analyzed.

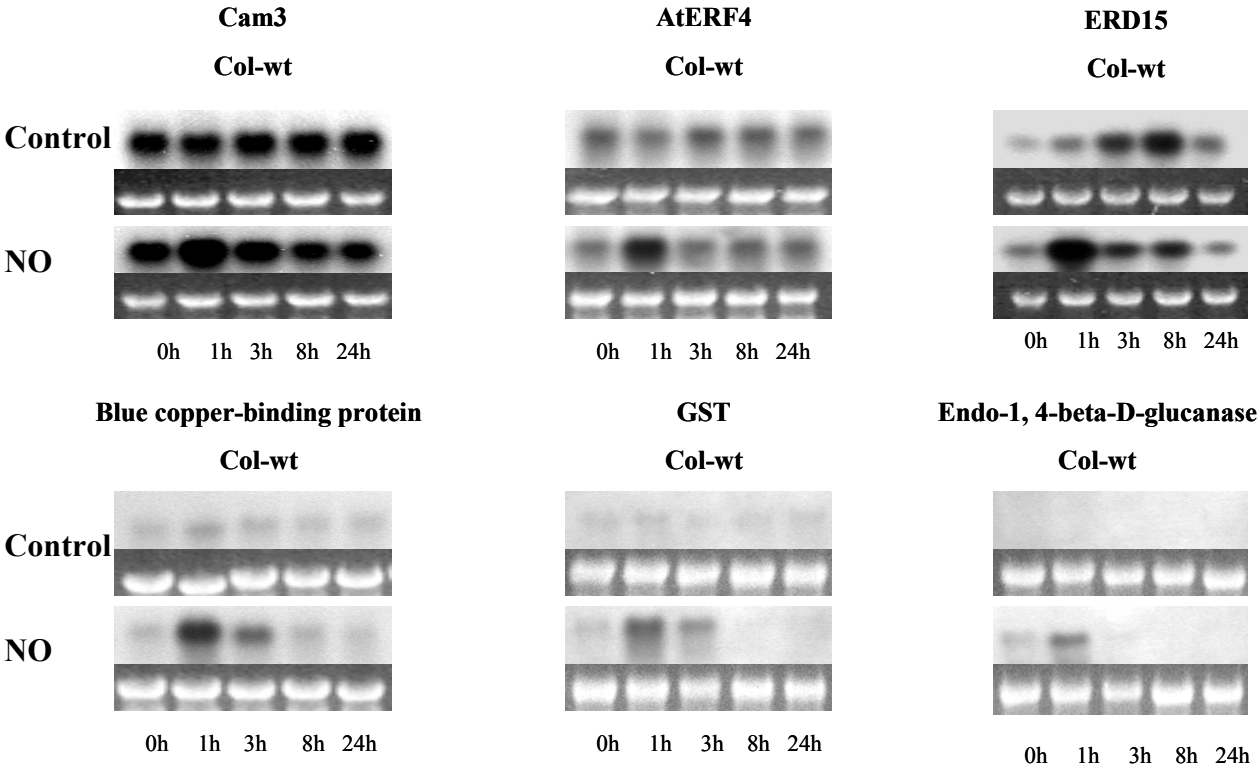


Figure 17: Gene expression in *Arabidopsis* wt plants after NO treatment. *Arabidopsis* plants were treated with gaseous NO (1250ppm) for 10 min. Leaves were collected at the times indicated for RNA preparation (0-24 h). Northern blots were probed with cDNAs for calmodulin 3 [CaM 3, At3g56800], AP2 domain containing protein RAP2.5 [AtERF4, At3g15210], dehydration-induced protein [ERD15, At2g41430], blue copper-binding protein [At5g20230], glutathione S-transferase [GST, At2g29420], and endo-1, 4-beta-D-glucanase [At4g30280]).

4.2. Microarray analysis of NO-induced gene expression in wt, jar1 and NahG plants

Salicylic acid and jasmonic acid signaling pathway are thought to be two most important pathways for plants defense response (Dong, 1998). NO is known to play a key role in plant defense systems. However, how does NO exert its function? Which genes are induced by NO? Moreover, how does NO cooperate with other signals to cope abiotic and biotic stresses? There are still many open questions. To unravel the interaction and/or participation of NO with known plant defense signaling pathways, gene expression dynamics was studied in NO treated *Arabidopsis thaliana* (Col-wt) plants by cDNA microarray. Identical experiments were carried out with NahG and jar1 plants. NahG plants contains bacterial salicylate hydroxylase

gene, which removes SA and prevents the establishment of SAR in plants (Gaffney et al., 1993). NahG is an optimal model plant to study SA signaling. Jar1 is a jasmonic acid resistant mutant screened by reducing sensitivity of root growth to methyl jasmonate and showing lower JA defense gene (such as AtVSP) expression after methyl jasmonate treatment(Berger, 2002). Jar1 was selected as model plants to study JA related gene induction.

A time course of NO-inducible gene expression was constructed in **Table 3**. For simplicity, only those genes for which the transcript levels changed substantially (only signals more than 2-fold above local background level and only expression ratios higher than 2.5) as a result of NO treatment are included (details see **Supplement Table S3**).

Table 3: Microarray analyses of transcripts in *Arabidopsis* wt, NahG, and jar1 plants.

	Function	Gene #	Col. wt				NahG				jar1			
			1h	SD	3h	SD	1h	SD	3h	SD	1h	SD	3h	SD
	Dehydration-induced protein (ERD15)	At2g41430	5,7	4,7	5,0	4,5	7,5	2,5	1,4	0,4	5,8	5,8	1,4	0,1
	Defense-related protein	At4g30530	3,0	1,9	2,5	1,8	6,4	3,8	2,3	0,5	3,9	3,3	1,2	0,1
	Cold-regulated protein kin2	At5g15970	7,6	1,3	6,0	0,2	2,5	1,6	2,2	0,6	3,5	1,7	3,0	0,3
	AtERF4	At3g15210	3,7	0,6	2,0	0,3	3,1	1,2	1,8	0,6	3,2	2,4	1,4	0,3
	Blue copper binding protein	At5g20230	20,0	10,5	8,7	0,7	17,6	11,2	3,6	1,2	12,2	4,5	5,2	0,7
	AOS	At5g42650	4,8	0,6	5,8	1,7	3,6	1,8	2,5	0,3	3,0	2,1	1,8	0,1
	CYP83B1	At4g31500	4,4	0,2	3,3	0,7	3,2	0,7	3,3	1,0	5,1	3,9	3,8	0,4
1	Non-race spec. disease res. protein	At3g20600	3,5	1,1	1,4	0,6	3,1	1,1	1,5	0,3	3,0	2,3	1,1	0,1
	Myrosinase - binding protein	At3g16470	1,8	0,5	3,7	1,9	3,1	1,4	5,7	1,5	1,7	0,6	7,9	1,3
	Glutathione S-transferase	At1g02920	4,8	0,5	5,0	1,9	3,3	0,3	3,1	1,1	3,1	3,1	2,7	0,2
	Polygalacturonase inhibiting protein 2;	At5g06870	5,0	1,4	3,7	1,2	4,3	1,4	3,0	0,5	3,1	1,8	3,6	0,3
	Xyloglucan endo-1, 4-beta-D-glucanase	At4g30280	7,6	3,5	1,5	0,4	2,6	0,5	1,4	0,3	3,0	1,9	1,3	0,1
	Glutathione s-transferase	At1g02930	5,2	0,7	4,8	2,0	7,5	4,5	4,3	2,0	3,2	3,9	2,2	1,5
	Glutathione s-transferase	At2g29420	5,3	2,1	2,3	1,0	11,2	10,6	3,0	1,0	3,9	3,0	1,3	0,0
	Anthranilate synthase beta subunit 9	At1g25165	4,1	1,3	2,5	1,1	3,1	0,9	2,7	1,0	2,9	1,4	1,9	0,2
	AOX1a	At3g22370	2,6	0,1	1,8	0,5	2,6	0,2	1,0	0,2	1,0	0,4	2,6	0,4
	Lipoxygenase 2	At3g45140	2,6	0,6	2,7	0,9	3,7	2,1	3,2	1,1	1,7	0,8	2,6	0,1
	Ubiquitin-conjucating enzyme	At5g41700	3,3	0,5	2,8	0,1	1,2	0,2	1,3	0,6	1,5	0,3	1,0	0,1
	Family II lipase EXL4	At1g75910	2,9	1,1	2,5	0,7	1,3	0,2	1,5	0,4	1,4	0,4	1,6	0,1
	Calmodulin-3	At3g56800	3,7	1,4	3,2	1,7	1,8	0,2	1,3	0,5	2,0	0,5	0,9	0,1
	Hypothetical protein	At3g47833	2,8	0,7	2,8	0,5	1,4	0,1	1,3	0,6	1,5	0,1	1,4	0,1
	Nitrate reductase	At1g77760	2,6	0,5	1,8	0,1	1,5	0,1	1,0	0,3	1,3	0,2	1,2	0,1
2	Peroxidase	At3g49120	2,7	0,1	2,0	0,5	2,5	0,5	2,0	0,1	2,0	0,6	2,1	0,2
	Mitogen-activated protein kinase 3	At3g45640	2,9	0,4	1,4	0,4	1,9	0,7	1,9	0,8	1,1	0,8	1,2	0,1
	ERE-binding factor	At5g61590	2,6	1,3	1,6	0,1	2,1	0,5	1,6	0,4	1,2	0,8	0,8	0,0
	S-adenosyl-l-homocysteine hydrolase	At3g23810	1,5	0,2	2,7	0,1	1,9	0,4	1,9	0,4	1,9	0,8	1,7	0,2
	Tryptophan synthase alpha subunit	At3g54640	2,1	0,4	2,9	0,9	1,7	0,3	1,0	0,3	1,0	0,4	2,4	0,1
	Nitrilase; NIT3	At3g44320	1,4	0,1	3,4	0,5	1,3	0,1	2,3	0,7	1,4	0,4	1,7	0,1
	Putative dicarboxylate diiron protein	At3g56940	1,0	0,1	0,8	0,2	2,8	0,9	2,4	0,8	1,8	0,9	1,0	0,1
	Putative nematode-resistance protein	At2g40000	0,3	0,1	0,4	0,1	2,9	0,9	1,2	0,1	1,3	0,7	0,5	0,1
	Cytochrome P450	At2g14100	1,6	0,2	1,7	0,3	2,3	0,9	2,9	0,9	1,7	0,7	1,1	0,1
	Low-temperature-induced protein 7	At5g52310	1,6	0,6	1,5	0,4	2,7	1,0	3,0	0,3	1,1	0,6	1,5	0,0
	PDF1.2	At5g44420	2,2	1,1	1,1	0,4	1,5	0,3	2,5	1,4	0,9	1,0	1,6	0,2
	AlphaDOX1 fatty acid dioxygenase	At3g01420	1,4	0,4	1,0	0,2	2,5	0,3	1,3	0,2	1,8	0,7	1,8	0,2
3	Jasmonate inducible protein	At3g16420	2,1	1,0	2,4	1,1	2,0	0,8	4,6	1,9	0,0	2,8	1,8	0,2
	S-adenosylmethionine synthetase 2	At4g01850	2,2	0,2	1,6	0,3	2,6	0,5	2,3	0,9	2,0	0,7	1,0	0,1
	Tryptophan synthase beta subunit	At5g54810	1,7	0,4	9,1	9,2	2,8	1,1	2,7	1,0	2,4	0,6	2,1	0,1
	Glutathione peroxidase; ATGP1	At4g11600	2,1	0,3	2,1	0,8	2,5	1,2	1,8	0,6	2,0	0,3	1,5	0,1
	Ethylene response sensor; ERS;	At2g40940	1,0	0,2	2,1	2,0	2,6	0,8	1,5	0,5	2,3	0,7	1,9	0,0

	Lipoxygenase 1	At1g55020	1,9	0,6	2,2	1,1	4,1	2,2	3,8	1,0	1,8	0,4	1,3	0,1
	Aldehyde dehydrogenase-like protein	At3g48000	0,8	0,0	0,8	0,2	4,0	3,3	1,9	0,7	1,4	0,3	1,1	0,1
	Vegetative storage protein (VSP2)	At5g24770	1,8	0,8	2,0	0,9	4,4	1,6	4,7	2,3	0,8	0,9	1,3	0,2
	Anthranilate synthase alpha subunit	At5g05730	1,7	0,6	1,6	0,6	4,5	1,7	4,7	2,3	1,8	1,2	1,1	0,1
	PR2	At3g57260	1,7	0,3	2,1	1,3	1,6	0,2	1,6	0,8	1,9	2,0	6,4	0,4
	PR5	At1g75040	1,4	0,2	2,0	1,4	1,4	0,2	1,1	0,5	1,8	1,9	2,8	0,2
4	Wall-associated kinase 2	At1g21270	1,5	0,3	1,4	0,6	2,2	0,3	1,5	0,4	2,8	0,8	2,1	0,3
	T14P8 unknown protein	At4g02340	0,8	0,1	0,7	0,0	2,4	0,9	1,8	0,7	3,3	3,2	1,0	0,1
	Glutathione S-transferase	At2g30860	1,0	0,1	0,9	0,0	1,6	0,4	2,3	0,6	1,7	0,4	3,7	0,3
	Vegetative storage protein (VSP1)	At5g24780	2,0	1,1	2,5	1,5	4,6	1,6	4,9	2,3	0,9	0,9	9,2	2,1
	Putative histidine kinase	At2g01830	1,6	0,7	0,9	0,5	3,8	1,0	1,7	0,5	2,7	1,7	1,4	0,1
5	Salt-tolerance zinc finger protein	At1g27730	1,3	0,8	1,1	0,7	2,6	1,0	1,6	0,5	2,5	1,3	1,3	0,1
	Arginine decarboxylase SPE2	At4g34710	1,6	0,3	1,3	0,1	3,2	1,4	1,7	0,1	1,3	0,8	2,6	0,2
	Aldose 1-epimerase-like protein	At3g47800	1,1	0,0	1,0	0,2	4,1	1,5	5,4	1,6	1,7	1,2	14,8	3,5
	Glutathione S-transferase	At2g30870	2,6	0,1	2,1	0,4	3,4	1,6	2,8	0,6	2,1	0,6	1,6	0,1
	Mac9 unknown protein	At5g61820	2,6	0,7	2,5	0,5	4,6	2,8	2,9	0,3	1,5	0,6	1,7	0,3
	Beta-1, 3-glucanase-like protein	At3g55430	3,2	0,4	2,6	1,0	2,6	0,6	1,7	0,5	1,5	0,4	2,0	0,2
	L-ascorbate peroxidase	At1g07890	3,0	0,4	3,7	1,3	2,6	0,6	2,0	0,7	1,8	0,4	1,6	0,1
6	MnSOD	At3g10920	4,1	0,1	3,5	1,6	3,0	1,2	2,1	0,5	2,2	0,9	1,4	0,1
	Nonexpresser of PR genes (NPR1)	At1g64280	3,2	1,1	1,5	0,8	2,8	0,7	1,6	0,6	2,3	1,2	1,2	0,1
	Pre-hevein-like protein;	At3g04720	4,5	0,8	3,4	1,9	1,8	0,3	2,6	0,9	2,2	2,1	1,5	0,1
	FAD7	At3g11170	3,2	0,1	2,6	1,1	4,0	1,9	2,5	1,1	2,4	1,0	0,8	0,1
7	PR1	At2g14610	1,5	0,3	3,2	2,5	2,2	2,1	2,2	0,3	4,0	4,8	9,2	1,0

Table 3: The genes with weak signals (lower than 2 fold local background) were neglected indicated by grey scripts. The genes with induction over 3.5 fold are marked by red colored boxes, and with induction between 2.5 - 3.5 fold by orange colored boxes. Array data were based on four independent replicates and the standard deviation (SD) is shown on the right side of each ratio.

The Venn diagram gives an overview on induced genes on the array after NO treatment of NahG and jar1 plants. The NO-inducible genes were classified into seven groups based on their expression pattern: (1) genes induced all in wt, jar1, and NahG; (2) genes only induced in wt; (3) genes only induced in NahG; (4) genes only induced in jar1; (5) genes induced both in jar1 and NahG; (6) genes induced both in wt and NahG; and (7) genes induced both in wt and jar1 (**Figure 18**).

Analysis of overlapping on the Venn diagram showed that 17 genes (group 1) were induced in all three plants. Apparently, induction of these genes did not depend on SA or JA. In case of GSTs and AOX (alternative oxidase), NO might directly feed into redox regulatory circuits (Huang et al., 2002). These results are consistent with previous observation on Northern blots (**Figure 11**).

In addition, the Venn diagram analysis shows that NO interacts with both JA as well as SA signaling pathways. On the one hand, 11 genes (group 2) are only induced in wt, indicating that these inductions are SA and JA signaling dependent. On the other hand, 13 genes are only induced in NahG plants (group 3), and 5 genes only in jar1 plants (group 4), which implied that the induction of these genes takes place only in absence, either SA or JA.

In the other cases (group 5, 6 and 7), these genes were induced only in two out of three tested plant types, which suggests that these NO-induced genes are dependent on the presence of certain signals (SA or JA). In the following section, I will try to sort out the induced genes by analysis of their functions.

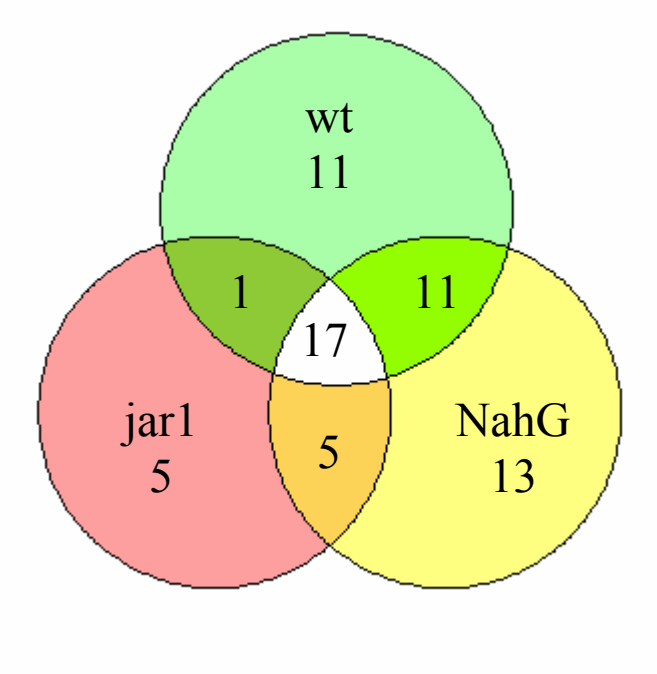


Figure 18: Classification of NO-inducible genes in *Arabidopsis* wt, jar1 and NahG plants. The NO-inducible genes were grouped in the following seven groups based on their expression pattern: (1) genes induced all in wt, jar1, and NahG; (2) genes only induced in wt; (3) genes only induced in NahG; (4) genes only induced in jar1; (5) genes induced both in jar1 and NahG; (6) genes induced both in wt and NahG; and (7) genes induced both in wt and jar1.

4.3. Classification of NO-induced gene based on their functions

Based on their physiological function, NO induced genes could be classified into several groups (**Table 4**): redox regulation, defense genes, transcription factors, JA signaling relative genes, and other genes (not shown in **Table 4**). However, alternative classifications are possible (Cheong et al., 2002).

As described in the previous section (**III 2.2**), NO induced oxidative stress and nitrosative damage in suspension cells. Gaseous NO induced many genes related to redox regulation including AOX1a, peroxidase, MnSOD, glutathione peroxidase, ascorbate peroxidase, and many glutathione S-transferases (GST) in plants. Peroxidase, MnSOD, glutathione peroxidase, and ascorbate peroxidase play key roles in regulation of reactive oxygen species

in plants (Neill et al., 2002b). The genome of *Arabidopsis thaliana* contains 48 GST genes. GSTs are predominantly expressed in the cytosol, where their GSH-dependent catalytic functions include the conjugation and resulting detoxification of herbicides, the reduction of organic hydroperoxides formed during oxidative stress etc. (Dixon et al., 2002). Induction of genes involved in redox-control suggests that NO treatment causes nitrosative or oxidative stress in plants.

The induction of many defense genes is not surprising, because NO has been known as an important regulator of defense response. Induction of pathogen-relative protein (PR1) is consistent with reported (Durner et al., 1998). It is reasonable that PR1 was not induced in NahG plants, because expression of PR1 is SA signal dependent and expression of bacterial salicylate hydroxylase in NahG plant prevents the accumulation of SA. Some of NO induced genes are not reported (e.g. non-race specific disease resistant protein, cold-regulated protein (kin2), low-temperature-induced protein 7 etc.). PR2 and PR5 were unexpected induced in jar1, but not in wt plant. Whether the constitutive JA inhibits the induction of them needs further identification.

As mentioned in **Introduction**, NO regulates gene expression by a multiple ways in animal system, from DNA methylation to posttranslational events. Well documented is the regulation of transcription factors. **Table 4** shows that NO induced several transcription factors in plants. The well known one is AP2 domain containing protein RAP2.5 (AtERF4), which has already listed in table NO-induced gene in suspension cells (**Table 2**). Another high interesting one is NPR1 (nonexpresser of PR genes). In mammalian system, NF-κB is best known for its stimulatory functions in the immune system, notably in B-cells and macrophages. It plays a key role in animal immune system (Stancovski and Baltimore, 1997). In plant, NPR1 is similar to mammalian I-κB, which regulates NF-κB (see described in **Introduction**). Elucidation the induction of NPR1 by NO may reveal the essential role of NO in plant immune system. This question was beyond the scape of this work.

Now, I focus on the following puzzling observations: (1) the key enzymes of the octadecanoid pathway, allene oxide synthase (AOS) and lipoxygenase (LOX2) were induced in Col wt, jar1 and NahG plants. (2) Nevertheless, in Col wt known JA responsive genes such as jasmonate induced protein (JIP) or the defensin PDF1.2 did not respond to NO, but in NahG plants, JIP, vegetative storage protein (VSP), and PDF1.2 were induced. To unravel the riddle, further identification is described in following section.

Other interesting induced genes are that of calmodulin 3 (CaM3), which is the most important protein for Ca^{2+} regulation and a key cofactor for NOS, and nitrate reductase and nitrilase,

which seem to take part in nitrate and nitrile metabolism, would be discussed detailedly in **Discussion**. Although most induced genes in this experiment are related to defense response, we cannot assert that NO induces mainly or only stress related gene, because the EST assembly for the experiment focus on stress genes.

Table 4. Classification of the NO-inducible genes based on their functions.

Classification	Function	Gene number	Expression patterns wt	NahG	jar1
Redox regulation	Glutathione S-transferase	At1g02920	+	+	+
	Glutathione S-transferase	At1g02930	+	+	+
	Glutathione S-transferase	At2g29420	+	+	+
	AOX1a	At3g22370	+	+	+
	Peroxidase	At3g49120	+		
	Glutathione S-transferase	At2g30860			+
	MnSOD	At3g10920	+	+	
	Glutathione peroxidase; ATGP1	At4g11600		+	
	L-ascorbate peroxidase	At1g07890	+	+	
	Glutathione S-transferase (ERD13)	At2g30870	+	+	
Defense genes	Defense-related protein	At4g30530	+	+	+
	Non-race spec. disease res. protein	At3g20600	+	+	+
	Xyloglucan endo-1,4-beta-D-glucanase	At4g30280	+	+	+
	Dehydration-induced protein	At2g41430	+	+	+
	Cold-regulated protein kin2	At5g15970	+	+	+
	Low-temperature-induced protein 7	At5g52310		+	
	PR2	At3g57260			+
	PR5	At1g75040			+
	Salt-tolerance zinc finger protein	At1g27730		+	+
	Beta-1, 3-glucanase-like protein	At3g55430	+	+	
	Pre-hevein-like protein;	At3g04720	+	+	
	PR1	At2g14610	+		+
Transcription factors	AtERF4	At3g15210	+	+	+
	ERE-binding factor	At5g61590	+		
	Ethylene response sensor; ERS;	At2g40940	+		
	Transcript factor inhibitor I kappa B	At1g64280	+	+	
JA signaling relative genes	Lipoxygenase 2 (LOX2)	At3g45140	+	+	+
	Allene oxide synthase (AOS)	At5g42650	+	+	+
	FAD7	At3g11170	+	+	
	Vegetative storage protein (VSP1)	At5g24780		+	+
	PDF1.2	At5g44420		+	
	Vegetative storage protein (VSP2)	At5g24770		+	
	Jasmonate inducible protein	At3g16420		+	

5. Identification of the role of NO in jasmonic acid signaling pathway

The induction of NO and ROS by pathogens seems to regulate plant defense responses and/or cell death (Delledonne et al., 1998; Delledonne et al., 2001; Durner et al., 1998). NO seems to feed into the well described SA-dependent signaling system. However, arrays data indicated that many JA related genes were induced by NO treatment. I was interested whether NO plays a role in other defense signaling pathways such as JA signaling, which, in part, is also acting through generation of downstream redox messengers such as H_2O_2 (Orozco-Cárdenas et al., 2001; Orozco-Cárdenas and Ryan, 1999). The following sections will endeavor to elucidate the role of NO in JA signaling pathway.

5.1. Verify the transcript accumulation of AOS and LOX2 by northern blot

Although cDNA arrays showed that AOS and LOX2 were induced in Col- wt, jar1 and NahG plants, the transcript accumulation of AOS and LOX2 were verified by Northern analysis, to ensure the reliability. The probes constructed for Northern hybridization were based on the primers used to generate the DNA for the array. To verify the array data on AOS expression, plants were treated with NO as described. Plants were harvested and total RNAs were extracted at the indicated time-points and subjected to Northern blot hybridization (**Figure 19**). The strong induction of AOS by NO confirmed the expression data obtained by the cDNA array (**Table 3**). Expression was transient and peaked between 1 and 3 h post treatment. To dissect the signal requirements for the expression of AOS, *Arabidopsis* mutants and/or transgenes with impaired signaling pathways were analyzed. I was predominantly interested in JA signal transduction mutants such as the JA-insensitive jar1 and jin1 and JA synthesis mutant opr3 (Berger, 2002; Stintzi and Browse, 2000). However, when treated with gaseous NO neither opr3, jar1 and jin1, nor the ethylene signaling mutation etr1 did influence the NO induction of AOS as seen in the wildtype (**Figure 19**). Furthermore, AOS induction by NO was not affected by the presence of the NahG gene. NahG plants express a bacterial salicylate hydroxylase, and accumulate little, if any, SA and, as a consequence, show reduced accumulation of SA-responsive gene transcripts.

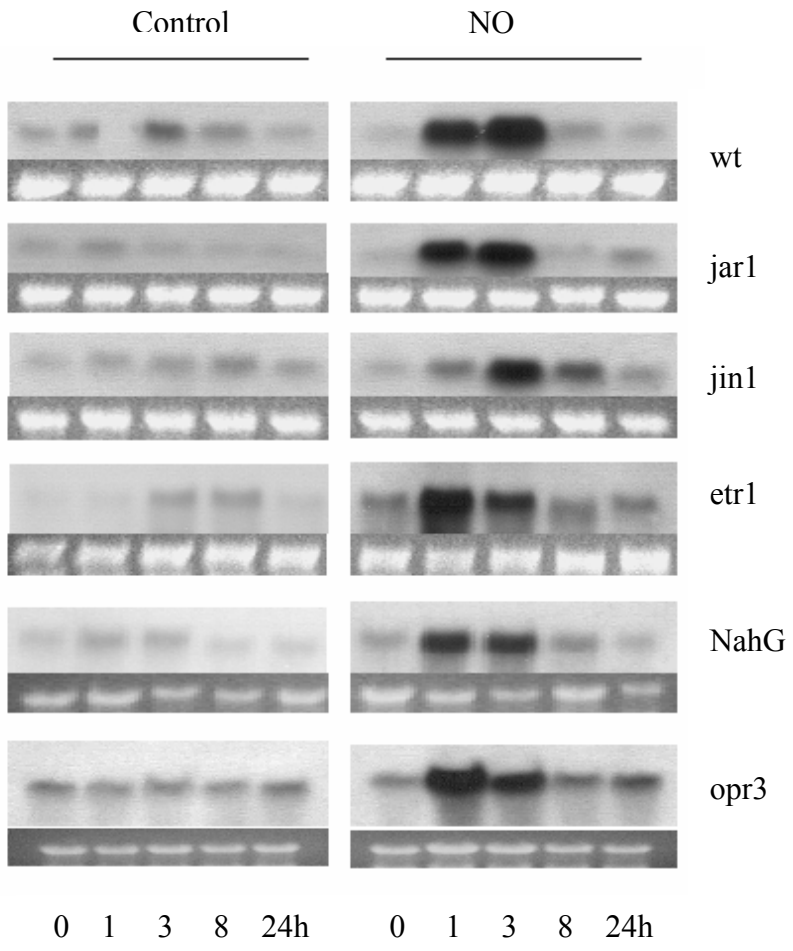


Figure 19: NO induces AOS gene expression in *Arabidopsis*. *Arabidopsis* plants (wild-type Col; the JA-signaling mutants jar1, jin1 and OPR3; the ethylene-signaling mutant etr1 and the transgene NahG, impaired in SA-signaling) were treated with NO as described. Leaf material was collected at the times indicated for RNA preparation (0-24 h).

To allow for an effective increase in pathway output capacity, the enzyme upstream of AOS, LOX2, is often induced in a coordinated manner (Farmer et al., 1998; Schaller, 2001). In my hands, kinetics of LOX2 expression paralleled AOS expression in the wild type and in the various signaling mutants (**Figure 20**). AOS and LOX2 show a similar expression profile after NO treatment and their induction seems independent on ethylene, SA or JA signaling.

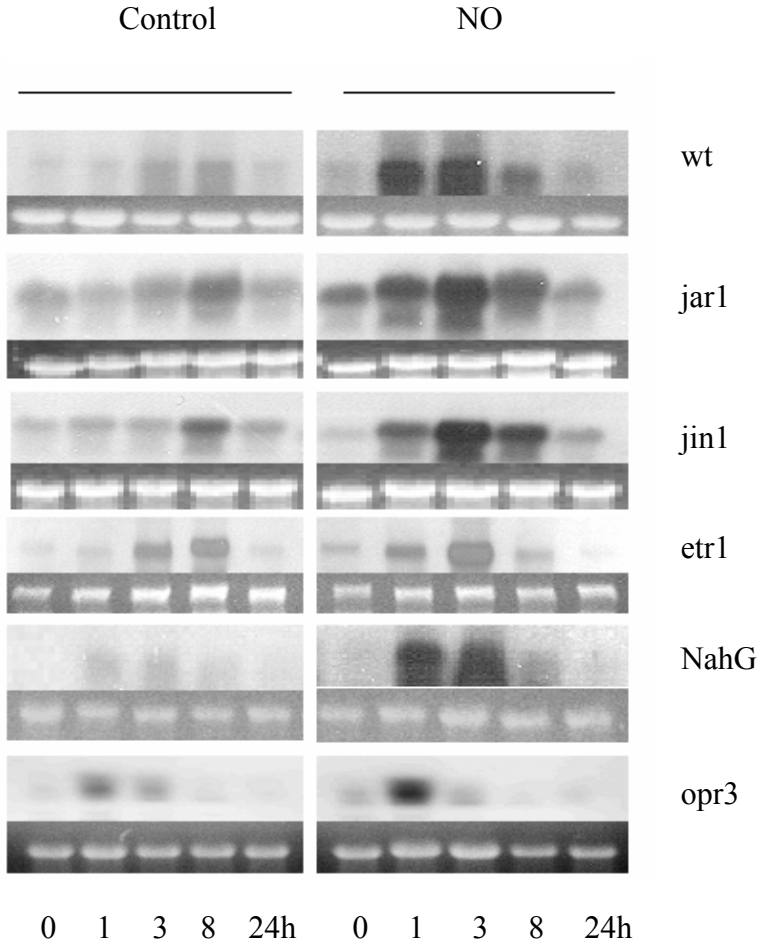


Figure 20: NO induces LOX2 gene expression in *Arabidopsis*. *Arabidopsis* plants (wild-type Col-wt; the JA-signaling mutants jar1, jin1 and opr3; the ethylene-signaling mutant etr1 and the transgene NahG, impaired in SA-signaling) were treated with NO as described. Leaf material was collected at the times indicated for RNA preparation (0-24 h).

5.2. cPTIO didn't inhibit the AOS induction by wounding

The induction of enzymes involved in JA biosynthesis such as AOS and LOX2 is considered to represent a key event of wound responses. Since NO leads to accumulation of AOS transcripts (**Table 3** and **Figure 19**), we asked whether NO plays a role in wound-induction of AOS. For this purpose NO production was suppressed *in planta* by infiltration of leaves with the cell permeable NO scavenger cPTIO. cPTIO did not influence AOS induction (**Figure 21**), so that during/after wounding transcription of *AOS* may be driven by additional factors other than NO.

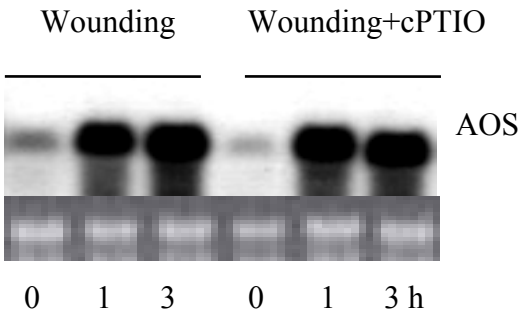


Figure 21: Expression of AOS after wounding does not depend on NO. *Arabidopsis* plants (wild-type Col) were infiltrated with 10 mM potassium phosphate buffer with or without 100 µM cPTIO, and subsequently wounded as described. Leaf material was collected at the times indicated for RNA preparation (0-3h). Northern blots were probed with cDNAs for AOS.

5.3. Induction of 12-oxo-phytodienoic acid reductase (OPR3) by NO

Besides AOS and LOX2, 12-oxo-phytodienoic acid reductase (OPR3) is another important enzyme involved in JA biosynthesis in *A. thaliana* (Stintzi and Browse, 2000), which does not exist on our EST assembly for microarray. There for, northern blot hybridization was carried out to analyze its transcripts after NO treatment. A specific probe was designed and used for transcript analysis (see **II 2.4.6**). Northern blots analyses indicated that OPR3 was induced in wt, NahG and JA mutants jar1 and jin1 plants by NO treatment, which are similar to the expression profile of AOS and LOX2 (**Figure 22**).

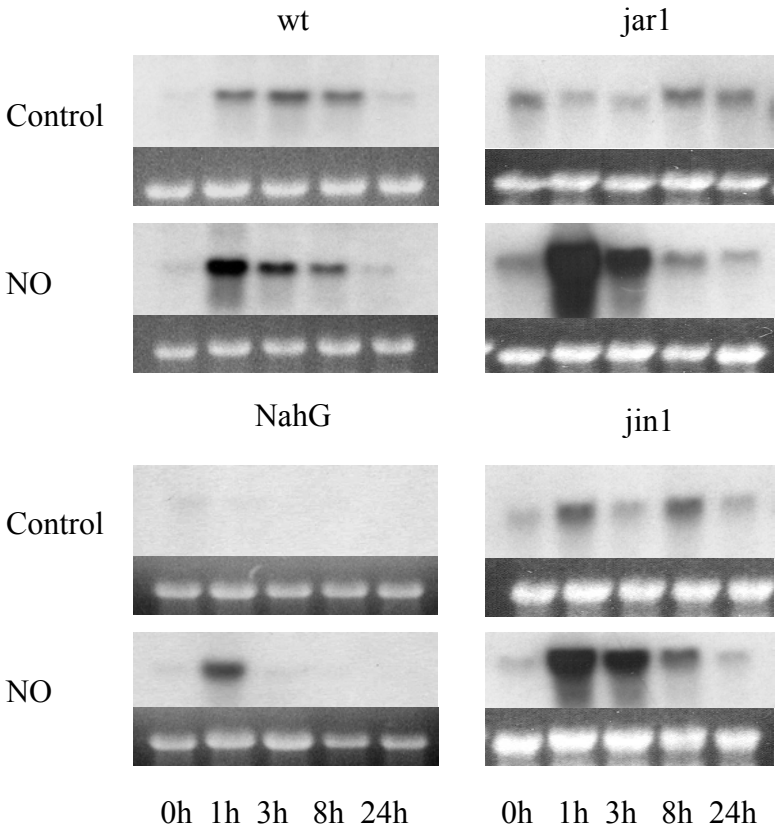


Figure 22: NO induces OPR3 gene expression in *Arabidopsis*. *Arabidopsis* plants (wild-type Col-wt; the JA-signaling mutants jar1and jin1; and the transgene NahG, impaired in SA-signaling) were treated with NO as described. Leaf material was collected at the times indicated for RNA preparation (0-24 h).

5.4. Analysis of defense genes downstream of JA

Array data indicated that despite the fact that NO-treatment of *Arabidopsis* was activating the JA biosynthetic machinery (**Table 3** and **Figure 19, 20,** and **22**) there was almost no accumulation of JA-responsive transcripts encoding defensins (PDF1.2) or jasmonic acid inducible protein (JIP) in NO treated wt plants. Surprisingly JA-responsible genes were induced in NahG plants (**Table 3**). To verify the array data, RNA from plants treated with NO was subjected to Northern blot hybridization. As previously reported by many others, JA led strong PDF1.2 accumulation (**Figure 23a**). Again, NO did not induce any PDF1.2 and JIP accumulation. Interestingly, when corresponding experiments were carried out with NahG plants, PDF1.2 and JIP, insensitive to NO in the wild-type, rendered NO-responsive (**Figure 23b**), which confirmed the array data. These data suggested a role of SA involved in suppression of JA- or NO-responsive genes.

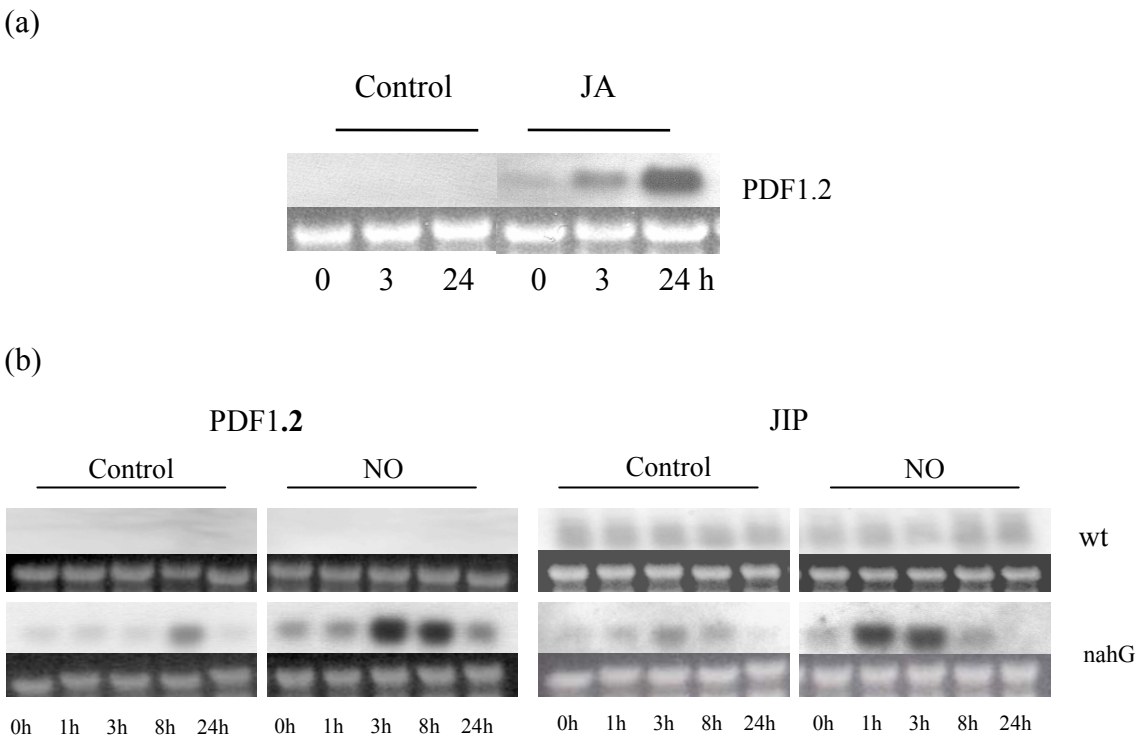


Figure 23: NO induces PDF1.2 and JIP gene expression in NahG *Arabidopsis*, but not in wild-type. (a) *Arabidopsis* plants (wild-type Col) were treated with 100µM JA. Leaf material was collected at the times indicated for RNA preparation (0-24 h), and analyzed for PDF1.2 expression. (b) Wt and transgenic NahG plants were treated with NO. Leaf material was collected at the times indicated for RNA preparation (0-24 h), and analyzed for PDF1.2 and JIP expression.

5.5. Determination of ethylene, SA and JA concentration after NO treatment

A link between NO action in plants and SA has been made previously (Klessig et al., 2000). NO seems to act, at least partially, through a SA-dependent signaling pathway. Treatment of tobacco leaves with NO induced a significant increase in the endogenous salicylic acid (Durner et al., 1998). In this work, NO induced AOS, LOX2 and OPR3 in wt and all mutants tested. NO induced JA defense genes such as PDF1.2 and JIP in NahG plants, but not in wt plants, which strongly suggest that the constitutive or NO induced SA suppresses the induction of JA -responsible gene. Direct quantification of free SA in leaves of NO-treated *Arabidopsis* plants revealed that NO was inducing SA biosynthesis. Moreover, NO treatment induced JA synthesis in NahG plants, but not in wt plants (**Figure 24**). These results imply that the increase of SA by NO treatment possibly inhibits the JA synthesis. As a result, the induction of JA downstreaming genes was repressed. Ethylene production was significantly reduced, which is consistant with previously reported (Leshem and Haramaty, 1996).

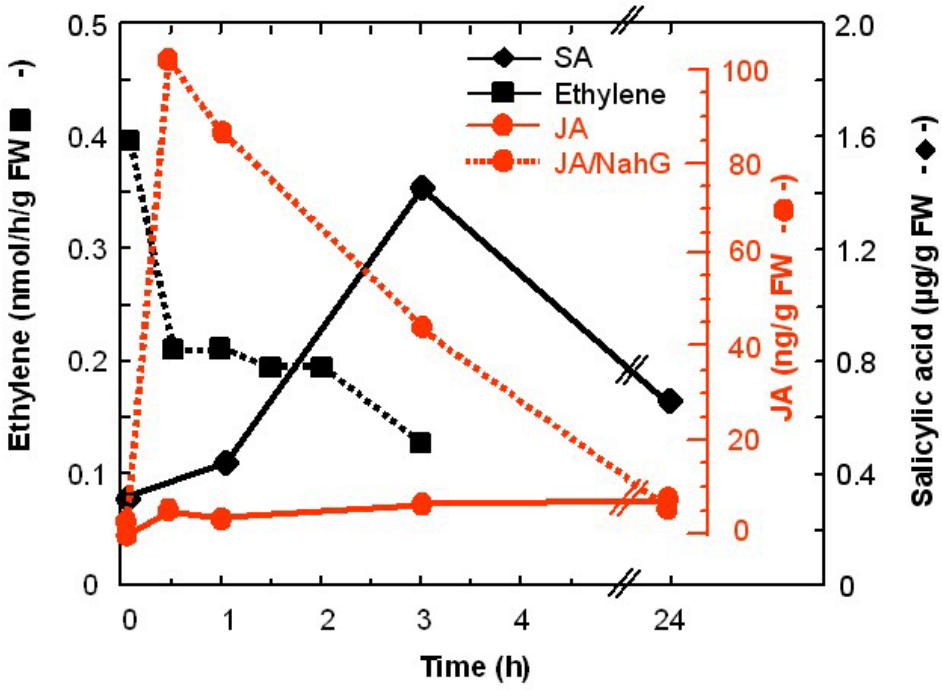


Figure 24: Effects of NO on SA, JA and ethylene concentrations in *Arabidopsis* leaves. NO treatment and determination of the levels of free SA, JA and ethylene were as described. Data are means of 2 or 4 (JA) independent experiments. For the sake of clarity the standard deviations are not shown in the figure.

5.6. DAF fluorescence detect the NO burst after wounding and JA treatment

Although NO was reported to negatively modulate wound signaling in tomato plants though inhibition of wound-inducible H_2O_2 (Orozco-Cárdenas and Ryan, 2002), my results showed that NO also positively regulated the JA synthesis gene such as AOS, LOX2 and OPR3, and also the JA responsive gene (e.g. PDF1.2 and JIP) in NahG plants. We were interested whether there is NO production during or after wounding. To answer this question, real-time imaging of NO by diaminofluoresceins (DAFs) was applied.

Real-time imaging of NO by diaminofluoresceins (DAFs) was done in conjunction with confocal laser scanning microscopy. DAFs do not react with any ROS, and lower the detection limit for NO to 5 nM. The diacetate derivative (DAF-2 DA) is used to load living cells, where it is hydrolyzed by cytosolic esterases to release DAF-2, which in the presence of NO is converted to the fluorescent triazole derivative (Kojima et al., 1998). DAF has been used to visualize NO production in Kalanchoe (Pedroso et al., 2000), barley (Beligni et al., 2002), mung bean (Lum et al., 2002) and tobacco (Foissner et al., 2000). The ability of diaminofluoresceins to specifically detect NO in biological systems has been confirmed (Suzuki et al., 2002).

Real-time imaging of NO production indicated that NO burst was induced in epidermal *Arabidopsis* cells by either wounding or JA treatment. Epidermal (abaxial) sections were loaded with DAF-2 DA and analyzed with confocal laser scanning microscopy (**Figure 25**). Wounding or addition of JA resulted in a rapid burst of fluorescence, indicative of a NO production. Fluorescence became visible in the cytosol and along the plasma membrane and/or cell wall. However, since DAF-2 DA is a single-wavelength probe, no adjustments can be made for differential accumulation of the probe in the cell. While there is no evidence for differential loading, DAF-2 DA or other fluorescein derivatives might preferentially accumulate in specific cellular compartments. The basal fluorescence shown in **Figure 25** represents basal NO. Scanning by using the laser (488 nm excitation) alone was ineffective in inducing measurable increase of fluorescence (data not shown). While DAF-2 DA is reported to be highly specific for NO (Kojima et al., 1998), we used a membrane permeable NO scavenger as a control. Carboxy-2-phenyl-4,4,5,5-tetramethylimidazolinone-3-oxide-1-oxyl (cPTIO) is highly specific for NO scavenging, does not react with any ROS (Barchowsky et al., 1999) and has been used to block NO production as well as NO-dependent cell death and defense gene activation in tobacco, soybean, *Arabidopsis* and barley (Beligni et al., 2002;

Delledonne et al., 1998; Durner et al., 1998; Foissner et al., 2000). 100 µM cPTIO completely suppressed both the basal fluorescence level as well as the elicited bursts of fluorescence.

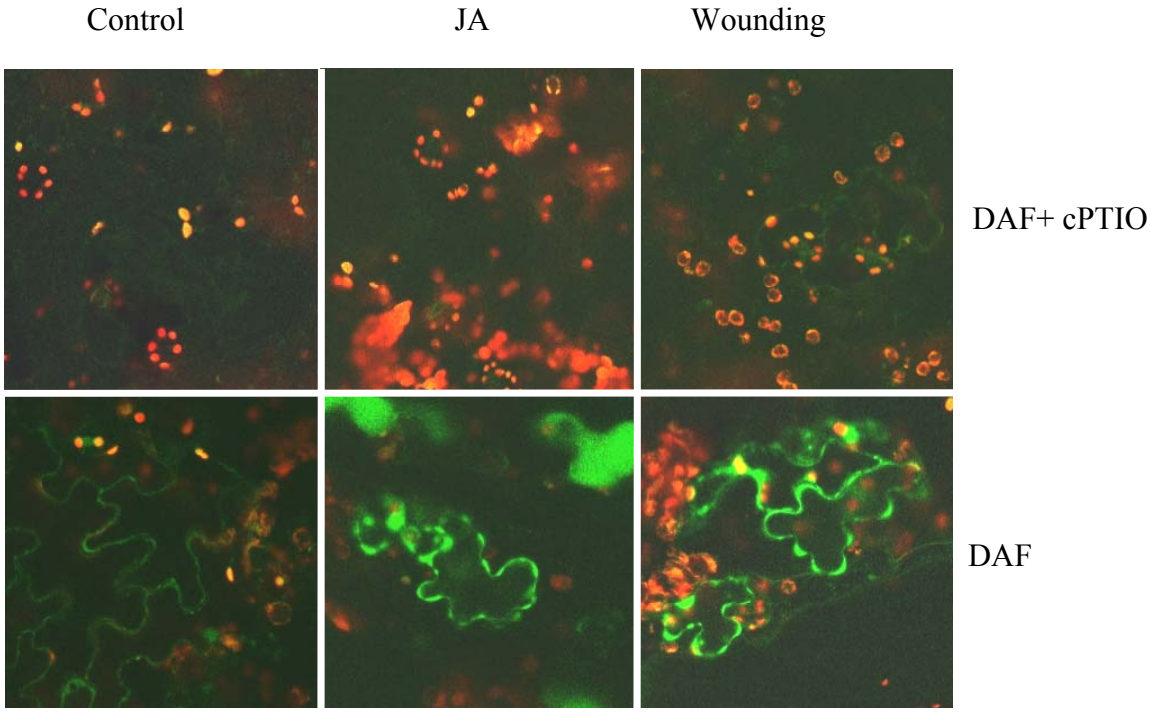


Figure 25: Confocal laser scanning microscopy of wounding and JA-induced increases in intracellular DAF-2 DA fluorescence in epidermal cells from *Arabidopsis thaliana* (wt). Plants were treated with JA (100 µM) or wounded as described. Epidermal peels were loaded with DAF-2 DA in absence (bottom row) or presence of the NO scavenger cPTIO (100 µM; upper row), washed, and examined under the microscope. The left column shows DAF-loaded, but otherwise untreated controls. The strong fluorescence in response to JA-treatment or wounding is indicative of NO-production, and could be suppressed by addition of cPTIO. Pictures were taken 10 min after wounding.

Corresponding results were obtained with epidermal peels from tobacco and the *Arabidopsis* JA signaling mutant jar1 (**Figure 26**; note that these pictures were obtained with a conventional fluorescence microscope). In case of wounded tobacco leaves, a strong NO production in stomatal guard cells (white arrows) and subsequent stomatal closure were observed. NO dependent ABA signaling in stomatal guard cells has been described for pea and bean leaves, respectively (Mata and Lamattina, 2001; Neill et al., 2002a).

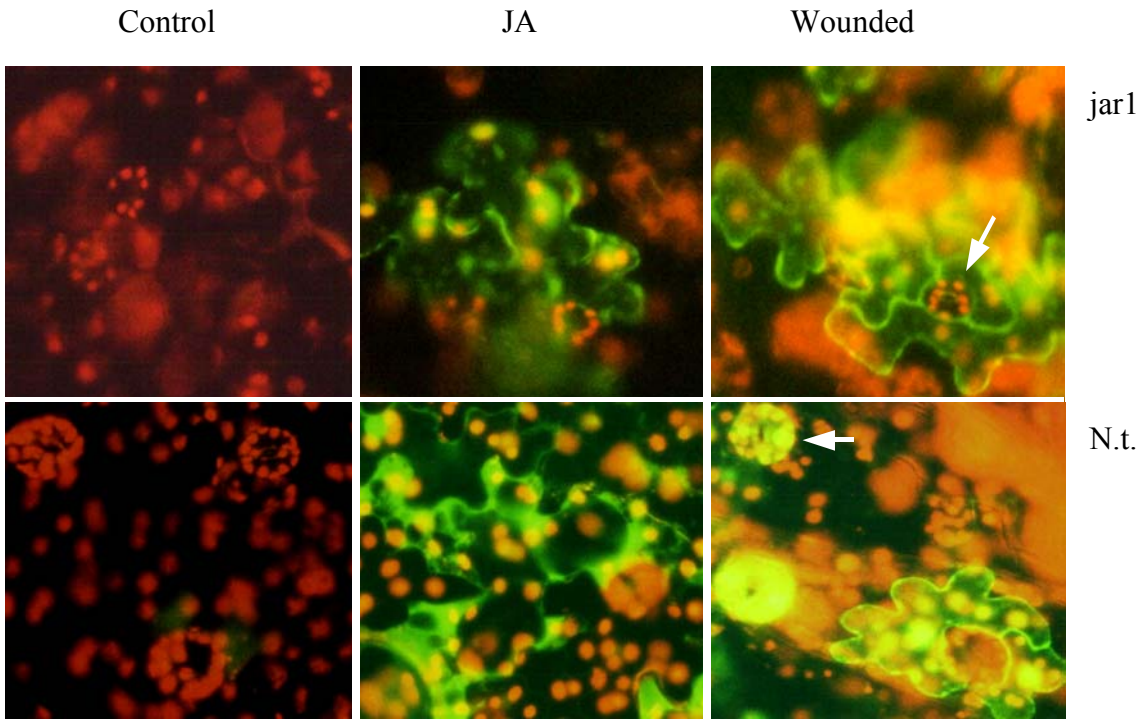


Figure 26: Wounding and JA-induced increases of intracellular NO in jar1 *Arabidopsis* mutant and in tobacco (*Nicotiana tabacum*). Plants were treated with JA (100 µM) or wounded as described. Epidermal peels were loaded with DAF-2 DA and subsequently examined by conventional fluorescence microscopy. The upper row shows jar1, the bottom row tobacco cells. The presented pictures were taken in absence of cPTIO. The white arrows point to stomatal guard cells, which in case of tobacco produce large amounts of NO.

5.7. Electron paramagnetic resonance (EPR) detect the NO burst after wounding

DAF fluorescence is proven as sensitive tool to real-time detect NO at nM level. To attain the solid evidence that wounding really induces NO burst in plants, an alternative method with more confidence was applied. Electron paramagnetic resonance (EPR) spin trapping is considered to be one of the most reliable approaches for direct detection of authentic NO in biological situations (Kalyanaraman, 1996). EPR detects NO with using ferrous and mononitrosyl dithiocarbamate ($Fe^{2+}(DETC)_2$) as spin trapping agent (Tsuchiya et al., 1996), which is able to detect the NO not only in animal cell (Kleschyov et al., 2000), but also in plant cells (Mathieu et al., 1998; Pagnussat et al., 2002).

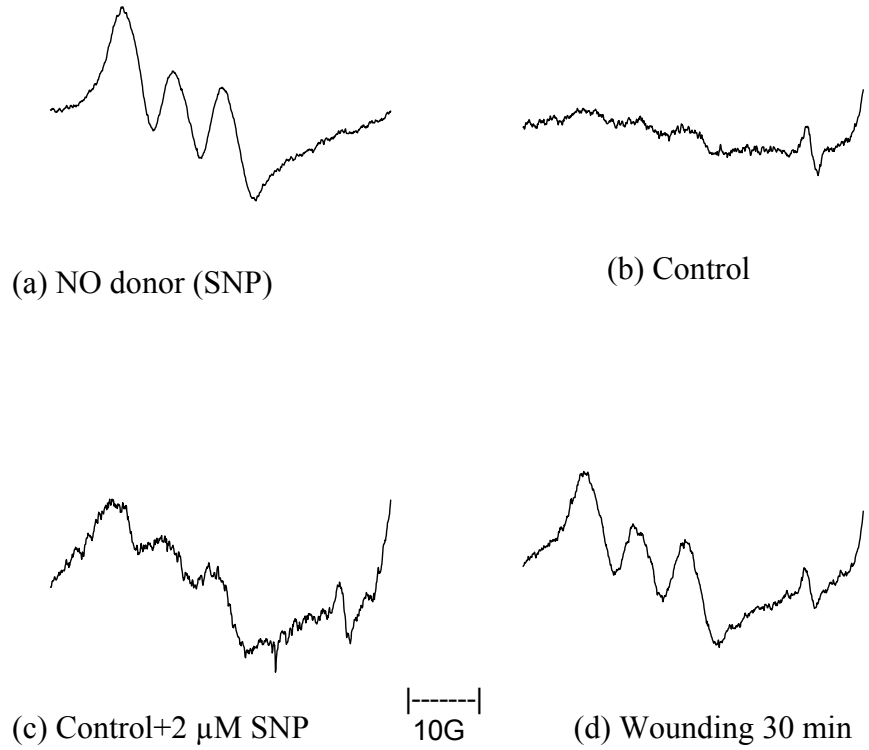


Figure 27: Wounding induces increase of endogenous NO in *Arabidopsis* plants determined by EPR. (a) Spectrum of NO donor SNP; (b) of controlled plant materials; (c) of controlled plants materials and 2 µM SNP; (d) of the plants materials harvested 30 min after wounding. Spin trapping reagent: 2M $Na_2S_2O_4$, 3.3 mM DETC, 3.3 mM $FeSO_4$ and 33 mg/ ml BSA.

Incubation of 10 µM NO donor SNP with spin trapping reagent exhibits the characteristic spectrum features of NO-$Fe(DETC)_2$ signal (**Figure 27 a**). No large amount, but basal NO production was detected in untreated plant materials (**Figure 27 b**). The EPR feature of control material with 2 µM SNP was largely, but not completely overlapped by EPR feature of SNP (**Figure 27 c**). An obvious spectrum peak could be found in all features with plants materials, which suggest that it is possibly a plant material specific spectrum peak. Wounding induced endogenous NO burst was detected by EPR in wounded plants materials with typical spectrum features of NO-$Fe(DETC)_2$ (**Figure 27 d**), which verified the detected signal using the permeable and specific NO-sensitive fluorophore 4,5-diamino-fluorescein diacetate (**Figure 25**).

5.8. NO primes for JA accumulation after wounding in *Arabidopsis* leaves

NO induced AOS, LOX2 and OPR3, but no JA accumulation and expression of JA defense genes in wt plants. A possible reason was that NO treatment didn't activate phospholipase D (PLD), which are indispensable for releasing linolenic acid, the substrate for JA synthesis from lipid membrane after wounding (Wang et al., 2002). Primary investigation also showed that NO treatment didn't change the transcripts of PLD (data not shown).

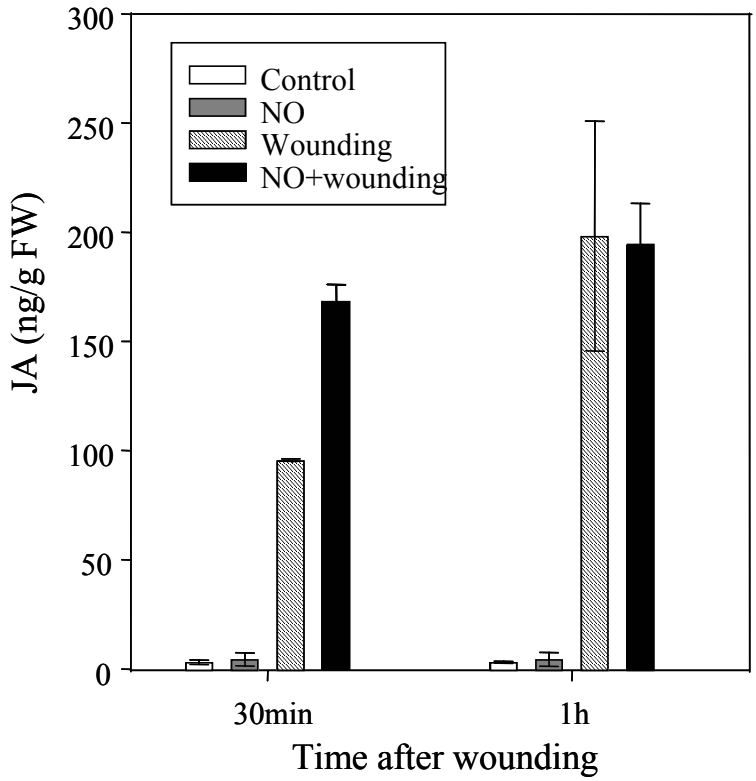


Figure 28: NO primes for JA accumulation after wounding in *Arabidopsis* leaves. *Arabidopsis* plants were /or not treated with gaseous NO, and then /or not wounded as described. Leaves were harvested at indicated time points and JA determination was done as described.

However JA and SA determination (**Figure 24**) and the PDF1.2 and JIP induction in NahG plants (**Figure 23**) suggested that the discrepancy between induction of JA synthesis machinery and no JA synthesis may be explained by the increase of SA and its potential antagonistic role. To reveal whether JA synthesis machinery was really activated by NO treatment, JA determination was carried out in *Arabidopsis* wt plants, which were treated with

NO, and then wounded 1h later. JA determination showed that NO treatment alone didn't induce JA accumulation. However, about 50% more JA accumulation was found in NO treated and wounded plants than in plants wounded alone at 0.5 h after wounding (**Figure 28**). It should be noted that the determination time point precedes the generation of reported downstream messengers of NO such as SA (**Figure 24**). Thus NO primes for JA accumulation after wounding, which suggests that the JA synthesis machinery was at least partly induced by NO treatment.

IV. Discussion

Nitric oxide (NO) is a multifunctional effector in numerous mammalian physiological processes, including the relaxation of smooth muscle, inhibition of platelet aggregation, neural communication and immune regulation. NO has also been associated with plant defense responses during microbial attack, and with induction and/or regulation of programmed cell death. In this work, I will try to reveal which genes could be induced in *Arabidopsis thaliana* by NO by using cDNA microarray.

1. DNA microarray

Before the beginning of this work, a working process and related protocols for filter-based radioactive labeling cDNA microarray have been set up in BIOP by work groups headed by PD Dr. Anthon Schaeffner and Dr. Dietrich Ernst. What encourages and prompts me to develop fluorescent labeling microarray were not only health concerns, but also the prominent advantages of fluorescent microarray.

1.1. Comparision of fluorescent and filter-based microarray

Compared to the macroscopie format of filter-based assays, the miniaturized biochip format represents a fundamental revolution in biological analysis. One advantage of the chip formats is that the solid surface is non-porous and thus enables the deposition small amounts of biochemical material in a precisely defined location. Porous substrates such as nylon and nitrocelluloses allow diffusion of applied material and are not amenable to microarray preparation. A non-porous substrate also prevents the absorption of reagents and sample into the substrate matrix, allowing the rapid removal of organic and fluorescent compounds during biochip fabrication and use. A non-porous surface permits the use of small sample volumes, enabling high sample concentrations and rapid hybridization kinetics. A solid substrate also provides uniform attachment surface, which increases the quality of the array elements. The inherent flatness of the microarray format permits true parallelism, which is lacking in all filter-based assays. Parallel analysis provides a significant increase in the accuracy of the assay data (Schena et al., 1996).

1.2. The nonlinearity in cDNA microarray expression measurements

Nylon membrane microarrays are hybridized with ^{32}P or ^{33}P-labeled cDNA targets, and the microarrays on glass are hybridized with fluorescent dye-labeled cDNA targets. After hybridization the radioactive or fluorescent signal intensities are measured using a phosphorimager or laser scanner, respectively. The signal intensities are surrogates for the expression levels of the genes in the samples under testing and are used to make biological interference. A key assumption in analysis of microarray data is that the quantified signal intensities are linearly related to the expression levels of the expression levels of the corresponding genes. At facts, both of the microarrays systems are not linearly. The first, which led to discrepancies in analysis affecting the fluorescent signals, was signal quenching associated with excessive dye concentration. The second, affecting the radioactive signals, was a nonlinear transformation of the raw data introduced by the scanner. Investigation of two types of nonlinearities showed that the second one is more troublesome (Ramdas et al., 2001).

1.3. Data analysis and normalization

The probes of nylon membrane microarray are ^{32}P or ^{33}P-labeled cDNA, which have to hybridize on two different membranes. How to ensure the same cDNA amount on two membranes is a prominent problem. A favorite strategy is hybridizing the membranes with ^{32}P or ^{33}P-labeled primer sequence to determinate and to normalize the amount of spotted cDNA (Thimm et al., 2001). However, the radioactive labeled primer for reference hybridization is not easily washed off completely, which results in radioactive residue on the membrane. In fluorescent labeled microarray, two probes (Cy3 and Cy5) can be hybridized to a glass slide and be detected by scanning with two wavelength (550 and 650 nm), that avoids the difference of DNA amount between two compared membranes.

As mentioned in **Methods** and **Results**, normalization is a key step in data analysis. Because NO is a multiple regulator of gene induction and NO-regulated genes are still less known, it is impossible to designate reference genes for NO treatment. Total fluorescence intensity was applied to data normalization. The shortcoming of this normalization is in bold relief. On the one hand, the 330 genes chosen for the experiments are not randomly selected, because I pay my attention on stress genes. On the other hand, the amount of genes is not large enough to ensure the up- and down- regulation in an approximate balance. The array results have

confirmed this consideration not surplus. Most of NO induced genes are up- regulated (**Tables 2** and **3**). I would suggest for future array analyses of NO treatment, to choose the control genes for normalization from **Table S2** and **S3**, which show lower regulation by NO treatment.

1.4. Confidence of the fluorescent labeling cDNA microarray

The filter-based radioactive labeling and glass-based fluorescent labeling cDNA microarrays were compared and the advantages of glass-based array were discussed. However, the most concerned is the confidence of the results. Fluorescent labeled arrays were introduced in BIOP for the first time, for which reason its reliability was payed much attention to. To ensure the confidence of the result, a strict standard was used for data analyses (see **Methods**) and a high number of northern blots were applied to verify the array results (**Figures 7, 11, 17, 19, 20,** and **22**). All northern blots confirmed the results obtained by microarray analyses. Solid evidences indicated that the arrays data are reliable.

2. Microarray analyses of gene regulation by NO

The basic research strategy of this work could be drawn by an “input” and “output” system. NO treatment was designated as the “input” factor. Plants or cell culture react to treatment by changing their gene expression levels, which were revealed by microarrays analysis. As “output” information, transcription changes indicate the hints of plant physiological and biochemical regulation. NO treatment initiated important and dynamic changes in gene expression. In this study, cDNA microarrays identified a number of *Arabidopis* genes for which I was unable to find reports of NO induction in the literature. The data also confirmed previous studies of other genes for which activation by NO was described (Durner et al., 1998). In response to NOR3 and gaseous NO, the transcript levels for many genes increased to maximum values 1 to 3h after treatment and then began to subside toward the baseline (**Figures 6** and **16**). The gene induction pattern in the suspension cell system was obviously different from that in the plant system.

2.1. Gene induction after NOR3 treatment in suspension cells

Table 2 exhibits all NO induced genes in suspension cells, which were revealed by DNA microarrays. DNA microarrays proved itself a high throughput vehicle for the exploration of gene expression profile.

Among the most interesting induced genes are several genes encoding for pathogenesis-related proteins (e.g. beta-1, 3-endoglucanase, and pre-hevein-like protein) and a number of enzymes involved in antioxidant processes (e.g. Glutathione peroxidase, catalases and GSTs). These inductions are interesting but not surprising. NO plays a key role in defense gene induction (Durner et al., 1998) and these antioxidant gene are important components in connection with reactive oxygen species (ROS). NO and ROS have important roles in the activation of defense responses against pathogen attack (Beligni and Lamattina, 2001; Bolwell, 1999; Durner and Klessig, 1999). The induction of pathogenesis-related genes by NO treatment implied that NO is an upstream signal for many defense genes. Coordinated induction of so many redox-related genes suggests that NO is involved in the complex redox regulation system in cell culture.

Metallothionein (AtMt1) and blue copper binding protein are another interesting NO induced gene group. Metallothionein is an important heavy metal-binding ligands and plays a key role in heavy metal detoxification and homeostasis in plant cells (Cobbett, 2002). Blue copper binding protein is an aluminum (Al) induced gene (Richards et al., 1998) and its induction ameliorate Al toxicity in *Arabidopsis* (Ezaki et al., 2001). As described in section **Introduction**, one of NO's functional chemical basics is that NO reacts with transition metals, which leads to the formation of metal-nitrosyl complexes and subsequent conversion of protein activity.

AP2 domain containing protein RAP2.5 (AtERF4) belongs to AP2/EREBP family of transcription factors. Proteins of this family contain a plant-unique DNA-binding domain called the AP2 domain, so named because it was first found in the *Arabidopsis* protein AP2 (APETALLA2) (Jofuku et al., 1994). In tomato, transcription factors of AP2/EREBP family (Pti4, Pti5, and Pti6) activate defense responses when they are expressed in *Arabidopsis* (Gu et al., 2002). AP2 element induction by NO may imply that NO possibly regulates the gene induction through AP2 family transcription factors.

2.2. Gene induction by gaseous NO treatment in plants

The identical microarray has been used to analyze the gene induction in wt, NahG and jar1 plants. The use of the jar1 and NahG mutants yielded several broad categories of NO-inducible genes (**Figure 18**). It is important to note that a gene defined as jar1- or NahG-dependent or -independent in this study might be controlled differently under other conditions, and I used these several categories only for the present study. Use of NahG transgenic plants and jar1 mutant enabled us to insight which gene inductions are SA- or JA-signaling dependent, thereby make it possible to sort out the route of NO signaling.

Venn diagram analyses indicate the gene expression pattern in wt and mutants plants. The expression of many genes is obviously altered by a direct or indirect effect of the loss of a functional jar1 gene or of expression of NahG gene. Concretely, the category of NahG-independent genes contains genes that were induced both in wild-type and NahG plants (e.g. AOS, AOX1a). Some genes were induced in NahG, but not in wt plants (e.g. PR1, MAP kinase). Some genes were induced only in NahG, but not in wt plants (e.g. PDF1.2. and jasmonate induced gene). The similar categories can also be found in jar1 mutant (e.g. PR2 and PR5 are only induced in jar1). Furthermore, some genes were induced in NahG and jar1, but not in wt plants (e.g. salt-tolerance zinc finger protein and arginine decarboxylatase SPE2), whereas, some genes were only induced in wt plants (e.g. calmodulin 3). These results merit further attention because they indicate that jar1 or NahG might play subtle roles as a positive or negative regulator of other signal pathways controlling NO-inducible gene expression. The different induction profiles by NO treatment in NahG and jar1 plants also imply the direct or indirect effects of SA and/or JA signals on NO signaling.

Next to the signaling-dependent arrangement, NO-induced genes could be classified into several categories by their physiological function (**Table 4**). Similar as described in suspension cell analysis (section **IV 2.1**), many defense genes and redox relative genes were also induced in plants by NO treatment, which implied the same meaning that NO plays a key role in plants defense response and that NO participates in redox regulation.

The list of NO-induced genes contains many previously reported genes. Besides PR1 (Durner et al., 1998; Huang et al., 2002), the induction of nitrate reductase (NaR) by NO is consistent with early report in tomato (Murray and Wellburn, 1985). Another important NO inducible plant defense gene is phenylalanine ammonia lyase (PAL) (Durner et al., 1998), which was not induced in my experiment. A possible explanation may be the different treatment

conditions, or too strict analyses standards which neglected the induced gene due to its weak signal intensity.

Interestingly, the transcript levels of NPR1 (**Table 3**) was induced in wt and NahG plants but not in jar1 plants. NPR1 gene has been implicated in the signaling pathway that leads to resistance against bacterial and fungal pathogens (Cao et al., 1997). Another interesting NO induced gene is calmodulin 3. A vast amount of cellular responses to external stimuli in eukaryotes involve second messenger Ca^{2+} signals. Transducing these signals, integrating their effects with those of other signaling pathways, and maintaining a homeostatic balance of Ca^{2+} to minimize its cytotoxic effects are initiated by a specialized group of cellular proteins, the EF-hand calcium-modulated proteins. Calmodulin (CaM) is the most widely distributed member of this protein family and is thought to be a primary intracellular Ca^{2+} receptor in all eukaryotes. The hallmark of CaM's mechanism of action is that it transduces second messenger Ca^{2+} signals by binding to and altering the activity of a variety of other proteins (Zielinski, 1998). CaM is also important cofactor for either mammalian or plants NOSs. Activity of constitutive NOSs is strictly dependent on the elevation of intracellular free Ca^{2+} and resultant binding of the Ca^{2+}-CaM complex (see **Introduction 2.1**). Moreover, NO has been proposed to induce defense response through a Ca^{2+} dependent second messenger pathway (Durner and Klessig, 1999). Up-regulation of CaM 3 by NO is either NahG or jar1 dependent (**Table 3**) and Northern blot also verified the array results (**Figure 17**). This finding confirms that NO is involved in Ca^{2+} regulation possibly through control calmodulin gene expression.

Another interesting group of genes encoding for enzymes responsible for JA synthesis (FAD7, LOX2, and AOS) are coordinately induced by NO treatment (**Table 4**). Moreover, JA downstream gene such as defensin (PDF1.2), vegetative storage protein (VSP), jasmonate inducible protein, were induced in NahG plants, but not in wt plants. All these results implied the subtle role of NO in the complex correlation with JA and SA signaling. Additionally, the concerted induction of a metabolic cassette implies tight control of expression among genes with potentially related functions and opens the door for further studies using other biochemical and physiological methods (further discussion see section **IV 4**).

2.3. Comparison of NO-dependent gene induction in suspension cells and in plants

Tables 2 and **3** show the gene induction by NO in suspension cells and in plants, respectively. The two biological systems show different gene expression profile after NO treatment. The gene induction in cell culture indicates intracellular, rather than intercellular signaling. NO is known as a freely diffusible inter- and intracellular messenger produced by a variety of mammalian cells (Aslan and Freeman, 2002) and no evidences indicate that NO is only a intracellular signal in plants. To study NO's intercellular role, transcripts analysis in whole plants is indispensable. In order to clarify which gene inductions are induced in cell culture alone and which gene inductions are intercellular signaling dependent, the NO induced genes in suspension cells and in plants (wt) were divided into three groups: (1) genes induced in cell cultures and plants, (2) only in cell culture, and (3) only in plants (**Table 5**).

The different expression patterns are possibly due to distinct manner of NO treatments (NO donor and gaseous NO). However, difference of expression pattern in cell culture and in plants may reflect the intracellular- and/or intercellular-signaling dependency. The arranged data show that most of the genes induced both in cell culture and in plants are redox regulation related (e.g. GSTs, peroxidase, and AOX1a). Evidently, some other redox related genes are induced only in plants or in cell culture.

The inductions of defense genes are also scattered. Some of them (pre-hevein-like protein, bata-1, 3-glucanase-like protein, blue copper binding protein) are induced both in cell cultures and plants, whereas, defense-related protein and non-race specific disease resistant protein were induced in plants and beta–glucanase 3 was induced in cell culture. This phenomena suggest that some defense genes and redox related genes seem to be intracellular inducible and some of them are intercellular signaling required.

Interestingly, AOS was induced in cell culture and in plants, whereas LOX2 and FAD7 were only induced in plants, which implies that the genes in the same signaling pathway may be regulated in a different manner. One could be regulated by intracellular pattern, whereas others by intercellular pattern. The different expression profiles may also be explained by different manner of NO treatment.

Table 5. Comparison of the genes induction in suspension cells and in plants (wt)

(1) Genes induced in cells and plants	(2) Genes induced only in cell	(3) Genes induced only in plants
Allene oxide synthase	5'-Adenylylphosphosulfate reductase (PRH43)	Dehydration-induced protein (ERD15)
AOX1a	Heat shock cognate 70kD protein	Defense-related protein
Ascorbate peroxidase	Cytoplasmic isozyme	Cold-regulated protein kin2
AtERF4	Glutathione-conjugate transporter	Non-race spec. disease res. protein
Beta-1, 3-glucanase-like protein	Isoflavonoid reductase homologue	Myrosinase-binding protein
Blue copper binding protein	Glutathione peroxidase (ATGP1)	Polygalacturonase inhibiting protein
GST (At1g02920)	pEARLI1	Xyloglucan endo-1, 4-beta-D-glucanase
GST (At1g02930)	PRH19 5'-adenylylsulfate reductase	Anthranilate synthase beta subunit 9
GST (At2g29420)	Seed imbibition protein	Transcription factor inhibitor I κB
Mac9 unknown protein	Peroxidase like protein	Ubiquitin-conjucating enzyme
Peroxidase	Auxin-responsive protein IAA10	Family II lipase EXL4
Pre-hevein-like protein	α-DOX1 fatty acid dioxygenase	Calmodulin-3
	Universal stress protein	Hypothetical protein
	Catalase (Cat1)	Nitrate reductase
	Beta-glucanase 3 (BG3)	Mitogen-activated protein kinase 3
	Polyubiquitin (ubq3)	ERE-binding factor
	Glutaredoxin-like protein (APR4)	S-adenosyl-l-homocysteine hydrolase
	Glutathione peroxidase 2 (GPX2)	Tryptophan synthase alpha subunit
	Flavanone-3-hydroxylase, F3H	Nitrilase; NIT3
	Chalcone flavanone isomerase	MnSOD
	Metallothionein (AtMt1)	Lipoxygenase 2
		FAD7

3. Identification the role of alternative oxidase (AOX1a) as NO counteracting principle

Most of the work on NO action in plant cells has focused on its ability to act in the same direction as reactive oxygen species (ROS). This concept explains NO participation in the hypersensitive response (Van Camp et al., 1998), and in the regulation of the expression of defense genes (Delledonne et al., 1998). However, in animals, where knowledge about NO biochemistry is more advanced than in plants, a toxic or protective role for NO has been widely described (Lipton et al., 1993). The presence of an unpaired electron within the NO molecule makes it a reactive species, and is also the origin of its duality. The combination of NO with ROS is described as either toxic or protective, depending on the circumstances (Beligni and Lamattina, 1999a). Induction of AOX1a by NO may relate to ROS and the toxic effect of NO.

3.1. Detrimental effect of NO

NO features an unmatched diversity of physiological functions and general ubiquity. Under pathological conditions, high concentrations of NO can be either beneficial (e.g. it activates defense responses or, together with ROS, it directly kills the pathogen) or detrimental. Among the deleterious effects are lipid peroxidation, oxidation of tyrosine as well as S-nitrosylation. Nitrosative stress can be linked to inhibition of cell growth and apoptosis, and thus may be widely implicated in NO pathogenesis. NO can therefore be considered a double-edged sword (Eu et al., 2000).

A key contribution to NO's toxicity and/or signaling properties is the fact that NO is a potent inhibitor of cellular respiration. NO's interaction with cytochrome c oxidase has dramatic biological consequences such as the induction of apoptosis (Brunori et al., 1999). The cause is not a shut down of respiration per se. In mitochondria, maintaining the ubiquinone pool at low steady state poise (i.e. avoiding over-reduction) is vital for minimizing the production of ROS. NO as an inhibitor of the cytochrome oxidase greatly enhances this thread, not only through inducing higher ROS production, but also because of NO's direct interaction with ROS. Many harmful effects of NO are actually mediated by peroxynitrite ($ONOO^-$), which is formed from NO and $O_2^{\cdot -}$ (Brüne et al., 1998; Hippeli and Elstner, 1996). Thus, it is no surprise that not only animal cells, but also *Arabidopsis* suspension cells react to NO treatment with induction of an array of antioxidant genes (**Table 2**). In kidney cells,

inactivation of glutathione peroxidase (GPX) by NO triggers a signal for inducing GPX gene expression in cells, thereby restoring the intracellular level of this indispensable enzyme (Dobashi et al., 2001). Strong induction of GPX, GSTs, glutaredoxins and other antioxidant genes could be seen. Surprisingly, neither catalases nor SODs were among the strongest induced genes (**Table 2**). The results point to a direct interaction between NO and the glutathione system as described for animals and discussed for plants (Durner and Klessig, 1999; Liu et al., 2001; Stamler et al., 1992).

3.2. The role of AOX in plant physiology

One of the most interesting genes induced by NO is AOX. In addition to enhance ROS scavenging principles upon NO treatment, plants may be able to reduce ROS-generation via induction of AOX. The plant cytochrome pathway is as sensitive to NO as its animal counterpart (Yamasaki et al., 2001). However, in addition to cytochrome oxidase, plant mitochondria possess AOX. Unlike the cytochrome pathway, which is coupled to oxidative phosphorylation via proton translocation, electron transport from ubiquinol to AOX is non-phosphorylating and releases energy as heat. These two terminal oxidases compete for electrons in plant mitochondria, and can be differentiated by inhibitors such as cyanide (acting on the cytochrome c oxidase) or SHAM (acting on AOX). Strikingly, it has been reported that AOX from mung bean and soybean, unlike cytochrome c oxidase, is also resistant to NO. Consequently, it has been hypothesized that AOX may play a role in the observed nitric oxide tolerancance of higher plants (Caro and Puntarulo, 1999; Durner and Klessig, 1999; Millar and Day, 1997; Yamasaki et al., 2001). **Figure 29** represents the plant respiratory chain in inner mitochondrial membrane and the possible role of AOX in maintaining the ubiquinone pool in a more oxidized state and preventing the generation of reactive oxygen radicals during nitrosative damage induced by NO.

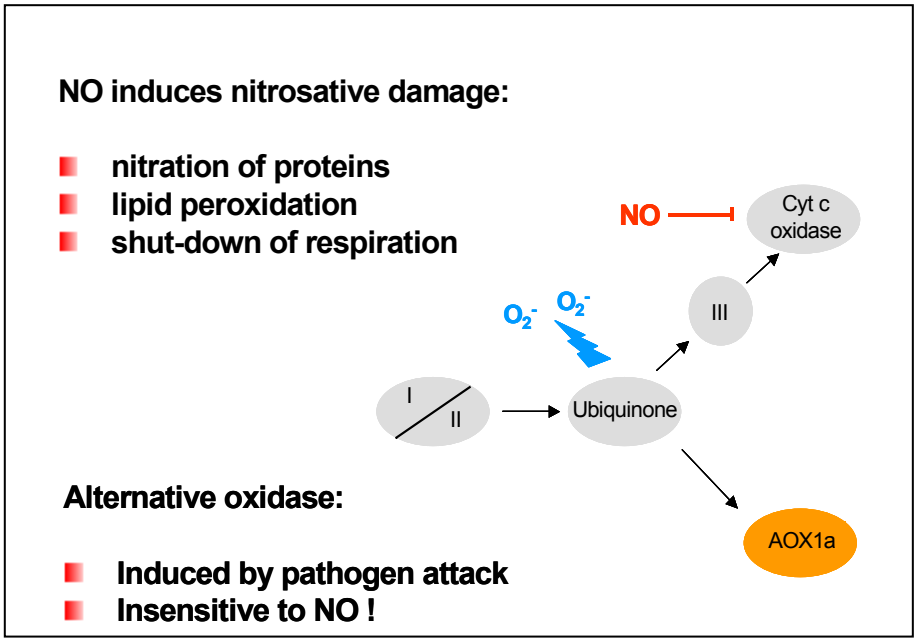


Figure 29: Diagram of the plant respiratory chain in the inner mitochondrial membrane. Electrons are passed from substrates such as NADH to ubiquinone, and then to cytochrome c oxidase or AOX. Over-reduction of the ubiquinone pool results in superoxide ($O_2^{\bullet-}$) formation. Nitric oxide (NO), or other inhibitors, such as cyanide, block cytochrome c oxidase and stimulate $O_2^{\bullet-}$ formation. Reaction between $O_2^{\bullet-}$ and NO generates peroxynitrite ($ONOO^-$). Operation of AOX will maintain the ubiquinone pool in a more oxidized state, preventing the generation of reactive oxygen radicals.

3.3. Induction of AOX by NO treatment

Considering the hypothesis of AOX counteracting NO, we speculated whether NO could actually have a stimulating effect on AOX activity and/or *AOX* transcription. In order to distinguish between the four AOX genes present in the *Arabidopsis* genome, we constructed specific probes as previously reported (Saisho et al., 1997). Here I show that NO treatment of *Arabidopsis* plants or cell cultures indeed induces *AOX1a* transcription transiently (**Figures 7** and **10**). There was a very weak basal expression of *AOX1*b, which could not be enhanced by NO. Transcripts of *AOX1c* and *AOX2* could not be detected, which is in line with previously published results on AOX expression in *Arabidopsis* leaves (Saisho et al., 1997). Expression of AOX1a was followed by an increased alternative respiration, which in turn was paralleled by a decrease of cytochrome c dependent respiration (**Figure 12**). This inhibitory effect of NO

on cytochrome c oxidase has been described for soybean and mung bean mitochondria (Millar and Day, 1996; Yamasaki et al., 2001).

We were interested in the signal requirements for the expression of *AOX1a.* Two lines of evidence pointed to salicylic acid (SA) as a signal involved in NO-mediated AOX induction: (i) the suggested cross talk between NO and SA signaling (Durner et al., 1998) and, (ii) SA has been demonstrated to induce expression of AOX in several plant species including voodoo lily and tobacco (Lennon et al., 1997; Raskin et al., 1987; Rhoads and McIntosh, 1993). It should be noted, however, that developmental regulation of AOX may not be solely dependent on SA (Lennon et al., 1997; Rhoads and McIntosh, 1992). To elucidate the potential participation of SA in AOX induction, I used *Arabidopsis* mutants which are not able transduce SA signals (*pad4* or *npr1*) or a transgenic plant that degrades any endogenously produced or applied SA (NahG). However, neither the SA signaling mutations *pad4* and *sai1*, nor the presence of NahG gene did influence NO induction of AOX (**Figure 11)**. These data suggest that NO induces *AOX1a* independently of SA. These results are in line with recent data on expression activation of *AOX1a* in *Arabidopsis* after *Pseudomonas* infection, which has been found to be independent of SA (Simons et al., 1999). It has been suggested that at least the coarse control of *AOX1a* seems to be directly connected to mitochondrial respiration, i.e. inhibition of cytochrome c respiration. How the status of mitochondrial respiration is perceived and transformed into *AOX1a* transcription and increased alternative respiration is unknown (Vanlerberghe and McIntosh, 1997).

3.4. The biological context of NO- induction of AOX

What might be the biological context in which NO-induction of AOX plays a role? In general, the alternative pathway may dampen the generation of active oxygen species during periods of rapid respiration (Maxwell et al., 1999). A most critical situation seems to be inhibition of respiration through NO, resulting not only in enhanced ROS pressure, but in simultaneous presence of NO and ROS. Most recently the combined action of NO and ROS during induction of cell death in soybean cells has been demonstrated (Delledonne et al., 2001). It has been suggested that such a scenario requires active AOX to maintain cellular function (Millar and Day, 1997) and **Figures 13, 14** and **15** demonstrate that while *Arabidopsis* cell suspensions can handle NO pressure or AOX inactivation, they don't tolerate NO without AOX. I am aware of the limits of pharmacological approaches. SHAM as well as other inhibitors such as n-propyl gallate may inhibit other enzymes involved in redox regulation

and/or alter the redox balance of the cell. On the other hand, I use AOX inhibitors within the documented concentration ranges, and without simultaneous application of NO no change in cell death or gene induction could be observed (**Figure 13**).

Conditions where NO is overproduced are nitrogen assimilation and pathogen attack. NO is a byproduct of assimilatory nitrogen metabolism, and NO production has been linked to the accumulation of NO_2 which is converted to NO by nitrate reductases or related enzymes (Kaiser and Huber, 2001; Stöhr et al., 2001; Yamasaki et al., 2001). AOX is closely linked to nitrate assimilation and, in *Chlamydomonas reinhardtii*, AOX was first identified as a nitrate assimilation gene *(Nar*5) (Quesada et al., 2000). AOX is resistant to NR-produced NO, supporting the hypothesis that AOX can play an important role in avoiding ROS accumulation during nitrate assimilation (Yamasaki et al., 2001).

AOX is also frequently induced during plant-pathogen interactions and plant defense, a situation in which NO is produced and plays an important role (Clarke et al., 2000; Corpas et al., 2001; Delledonne et al., 1998; Durner et al., 1998; Pedroso et al., 2000). NO is involved in cell death and defense gene activation where it acts together with ROS (Delledonne et al., 2001). The participation of peroxynitrite in plant defense and/or cell death has been shown (Alamillo and García-Olmedo, 2001). Others, however, reported on a protective role of NO counteracting ROS (Beligni and Lamattina, 1999b). AOX induction is coupled to plant defense, at least in many cases (Chivasa and Carr, 1998; Chivasa et al., 1997). Infection of *Arabidopsis* with *Pseudomonas syringae pv tomato* resulted in enhanced expression and activation of the AOX (Simons et al., 1999). After TMV infection of tobacco, AOX protein increased significantly (although electron-flow partitioning did not) (Lennon et al., 1997). AOX seems to play a role in containment of lesions and control of initial plant defense reactions (Chivasa and Carr, 1998). In all these cases, the induction of AOX seems be an integral part of defense responses. NO seems to be a good candidate for AOX induction. The balance between ROS generated in mitochondria and NO might be an important factor in cell death induction, and together with the induction of antioxidant genes (**Table 2**), NO-regulated *AOX1a* induction might be part of NO's redox-regulating properties.

In conclusion, NO and ROS play important roles in animal immunity and pathogenesis. A growing body of evidence suggests that NO and ROS also control plant development, growth and pathogen resistance. While many targets and/or effector molecules of NO might be identical in animals and plants, only plants counteract NO through the induction of AOX genes. NO-induced AOX induction might play a role during situations of enhanced NO-production such as nitrogen assimilation and pathogen attack. Ironically, AOX is found not

only in plants, but also in plant and animal pathogens such as *Trypanosoma brucei, Plasmodium falciparum* and *Septoria tritici* (Chaudhuri and Hill, 1996; Clarkson et al., 1989; Joseph-Horne et al., 2001). AOX in parasites or fungi may be important for the ability of the pathogens to avoid the host's NO-mediated immune response (Millar and Day, 1996).

4. Identification the role of NO in JA signaling pathway

4.1. Plants defense signaling pathways

SA has long been known to play a central role in plant defense response against pathogens. SA levels increase in plant tissue following pathogen infection, and exogenous application of SA results in enhanced resistance to a broad range of pathogens (Ryals et al., 1996). SA is required for SAR. But characterization of SAR in a variety of plant species has suggested the existence of a complex signaling network that involves many factors affecting various aspects of general disease resistance. It has become evident that SA-mediated SAR is not the only pathway that can lead to broad-spectrum disease resistance. Besides SA-mediated SAR, both jasmonic acid (JA) and ethylene have been shown to be important for these alternative responses, e.g. against wounding and insect attack (Doares et al., 1995). The wounding-induced octadecanoid pathway results in the synthesis of signal molecule JA and subsequent activation of defense gene (e.g. proteinase inhibitor gene) (León et al., 2001; Schaller, 2001). The role of ethylene in plant defense is somewhat controversial as it contributes to resistance in some interaction (Norman-Setterblad et al., 2000; Thomma et al., 1999) but promotes disease production in others (Bent et al., 1992; Hoffman et al., 1999; Lund et al., 1998). JA and ethylene signaling pathway are both required for induction of induced systemic resistance (ISR) (Pieterse and Van Loon, 1999).

4.2. Interaction of plants defense signaling pathways

Plant defense responses are regulated through a network of signaling pathways that are known to involve at least three endogenous plant signaling molecules: SA, JA and ethylene. There is growing body of literature suggests that these signaling pathways do not function independently. Rather, they are involved in a complex signaling network in which the different pathways influence each other through positive and negative regulatory interactions (Kunkel and Brooks, 2002). The cross-talk between signaling pathways leading to inducible defense gene expression has been and is still a topic of intense research. The regulators SA, JA and ethylene affect and control each other. The well known example is the mutual antagonistic relationship between SA and JA pathways. Aspirin and related hydroxybenzonic acids inhibit wound response and wound-induced gene expression in tomato plants (Doherty

et al., 1988; Pena-Cortés et al., 1993). Studies in tobacco reveal that JA inhibits the expression of SA-defendant genes (Niki et al., 1998). This antagonistic action has been proposed to be central to the plant's ability to fine-tune the induction of plant defenses in response to different plants pest and pathogens (Kunkel and Brooks, 2002). There is also limited evidence for positive interaction between the SA and JA signaling pathway. Results from early experiments with tobacco indicate that SA and JA act synergistically to induce PR1b expression (Xu et al., 1994).

4.3. The role of NO in JA signaling pathway

JA and SA are now regarded as global signals for defense gene activataion (Reymond and Farmer, 1998). Another current opinion is that SA and ethylene activate the cell death pathway induced by microbial attack or abiotic stress such as ozone, and that JA is necessary for proper lesion containment and modulation of SA-mediated cell-death. It has been suggested that NO and ROS play a major regulatory and/or executive role in these cell death events (McDowell and Dangl, 2000; Van Camp et al., 1998). ROS and JA are also messengers in wound responses. In tomato, H_2O_2 generated in response to wounding can be detected at wound sites and in distal leaf veins within 1 hr after wounding (Orozco-Cárdenas and Ryan, 1999). The close interaction of NO with defense-associated ROS induced by microbial attack prompted us to ask for a possible cross-talk of NO with wounding and JA signaling. My data obviously extent the field of vision of NO's role in wounding and JA signaling pathway.

4.3.1. Wounding induces NO burst in plants

First, using NO sensitive fluorophore DAF-2 DA in conjunction with confocal laser scanning microscopy, the NO burst was detected in epidermal cells of wounded *Arabidopsis* and tobacco leaves. This observation was verified by electron paramagnetic resonance (EPR) analysis (**Figure 27**). The kinetic of NO production was similar to an elicitor-induced NO burst in tobacco or mechanical stress of various gymnosperms (Foissner et al., 2000; Pedroso et al., 2000). Thus, the induction of NO seems to be an extremely early wounding response. While H_2O_2 generated in response to wounding can be detected at wound sites and in distal leaf veins of tomato within 1 hr after wounding, the response maximizes at about 4-6 hr in both wounded and unwounded leaves, and then declines. The time course of wound-inducible

H_2O_2 in *Arabidopsis thaliana* leaves was similar to that found in tomato (Orozco-Cárdenas and Ryan, 1999). Since methyl jasmonate (MJ) as well as JA-inducing signals such as systemin, chitosan, and all induce the accumulation of H_2O_2 in leaves (and possibly also NO), we asked whether JA treatment could trigger the NO burst. JA treatment resulted in strong NO production, even in mutants defective in JA signaling (**Figures 25** and **26**). NO production as response to wounding is not limited to *Arabidopsis*, but can be also observed in tobacco (**Figure 26**), and possibly other plants species.

4.3.2. NO induced JA signaling related genes

To elucidate NO's integration into or interaction with known signaling pathways (namely SA and JA signaling), gene expression dynamics in NO treated *Arabidopsis thaliana* plants were studied by using a bouquet array that included about 330 defense-related genes.

In wild-type plants, NO induced (not unexpectedly) several pathogenesis-related proteins and an array of anti-oxidant genes (**Tables 3** and **4**). The strong influence on the redox-regulating systems might be due to the fact that many harmful effects of NO are actually mediated by peroxynitrite ($ONOO^-$), which is formed from NO and $O_2^{\cdot -}$ (Brüne et al., 1994; Hippeli and Elstner, 1996). Strong induction of ascorbate peroxidase, SOD's, and many GSTs could be found.

One of the most interesting genes induced by NO was allene oxide synthase (AOS). AOS is of particular importance in biosynthesis of JA. The enzyme catalyzes the first reaction specific to the pathway, the dehydration of 13(S)-hydroperoxylinolenic acid to 12,13-epoxy-linolenic acid. AOS transcript levels as well as AOS polypeptide levels rise after mechanical stimulation, elicitation or wounding (Schaller, 2001). Thus, AOS is regarded as a central point of control of the octadecanoid biosynthetic pathway. In addition, NO did also induce lipoxygenase, LOX2, which is also involved in the wound-induced biosynthesis of JA (Schaller, 2001). However, induction of AOS (and LOX2) during wounding does not depend on NO (**Figure 21**). NO does not seem to be a key player during wounding responses, but a modulator (see below).

Surprisingly, despite the strong activation of the biosynthetic enzymes for jasmonates typical JA-inducible genes such as defensins or JIP's were not induced in wild-type *Arabidopsis*. Although cDNA arrays have become a robust and reliable tool, transcript accumulation of AOS, LOX2, PDF1.2 and JIP were monitored by Northern analysis. The Northern results confirmed the array data: no PDF1.2 and JIP despite high levels of AOS, LOX2 (**Figures 19,**

20 and **23**). Analyses of signaling molecules in NO treated *Arabidopsis* provided a likely answer. NO treatment did not result in elevated JA levels (**Figure 24**). These results are consistent with published data that suggested, it was that the output of the jasmonate pathway appears to be strictly limited by substrate availability. Overexpression as well as knock-outs of AOS do not alter the basal level of JA in unwounded plants (Laudert et al., 2000; Park et al., 2002). In my hands, NO-treatment did not alter the expression of phospholipase (D), the key enzyme feeding lipids into the LOX2/AOS pathway. It was speculated that rather than inducing JA itself, NO might prime plants for faster JA production and/or JA-dependent gene induction. In preliminary experiments, NO-treated plants showed a only a slightly faster JA accumulation after wounding. The biological significance of such a priming effect remains to be determined.

4.3.3. SA plays a role in inhibition of JA synthesis

In addition to substrate limitation, JA biosynthesis seems to be under control of other factors such as SA. The relationship between the SA and the JA/ethylene defense response pathways is not well understood. Some studies have demonstrated that these signals work synergistically to induce defense responses. However, other evidence suggests that these pathways function antagonistically (Farmer et al., 1998; Glazebrook, 2001). Plants responding to a given pathogen usually do not activate both SA-associated and JA/ethylene-dependent defenses. SA and JA have been shown to antagonize the activation of each other's defense responses, and SA can inhibit JA biosynthesis (Doares et al., 1995). Both pathways share signaling components that are involved in the positive and/or negative cross-regulation of their activities (Glazebrook, 2001; Van Loon et al., 1998). Since NO has been shown to induce SA biosynthesis in tobacco (Durner et al., 1998), I was interested in whether NO-treatment was activating SA biosynthesis in *Arabidopsis*. **Figure 24** demonstrates a significant increase of SA in plants exposed to NO. Furthermore, NO-treatment of NahG plants resulted in activation of PDF1.2 and JIP (**Figure 23**). These result are in contrast to observation made in tomato, where NO was still effective in proteinase inhibitor suppression after wounding even in NahG plants and where NO did not elevate endogenous SA levels of wild-type plants (Orozco-Cárdenas and Ryan, 2002). On the other hand, there are several reports on principal differences of signals involved in wounding responses between *Arabidopsis* and solanaceae (León et al., 2001; Ryan, 2000). In addition, Ryan and coworkers fed SNP through petioles of tomato, a compound that releases a downstream product of NO with different modes of

action, nitrosonium anion (NO^+) (Wendehenne et al., 2001). It is certainly possible, that nitrosonium acts as an antioxidant as suggested for other experimental systems (Beligni et al., 2002). If this is the case, NO (or NO^+) could suppress the H_2O_2-induced activation of proteinase inhibitor genes as suggested (Orozco-Cárdenas and Ryan, 2002). Nevertheless, these data confirm that NO is a negative regulator of JA-responsive genes such as defensins, albeit through a different mechanism, namely elevated levels of SA. Considering the pleiotropic nature of NO in biological systems, additional mechanisms (apparently operative in tomato) cannot be excluded.

4.3.4. Cross-talk among NO, JA-, and SA- signaling

Evidence has been provided for a cross-talk of NO with jasmonate signaling. This interaction might play a role in two different biological contexts. Several recent publications all demonstrate the participation of NO or peroxynitrite in programmed cell death, gene regulation and defense responses during microbial attack (Alamillo and García-Olmedo, 2001; Delledonne et al., 2001). In some hypothetical models H_2O_2, SA, NO and cell death are linked in a self-amplifying process, termed the oxidative cell death cycle (Van Breusegem et al., 2001). If NO regulates the JA/ethylene system, the model of an oxidative cell death cycle (cell death induced by NO and ROS) needs to be modified. Ethylene, the death-promoting signal, is downregulated by NO (**Figure 24**), and JA is regarded to be an inhibitor of cell death (Beers and McDowell, 2001). If NO works together with ROS towards induction of cell death, and at the very same time NO has the potential to stimulate the death inhibiting JA synthesis, NO would occupy a central role in the apoptosis regulating machinery just as reported for animals.

In case of wounding, NO seems to act as a dual modulator of JA-signaling. NO is certainly no key player of wound responses (**Figure 21**). However, I would like to extend the suggestion of Ryan and coworkers who regard NO as a negative regulator of wound signaling (Orozco-Cárdenas and Ryan, 2002). To put all results in a good order, NO's effects on JA signaling pathway were schematically represented (**Figure 30**). Both wounding and JA induce a NO-burst, and NO stimulates AOS, LOX2 and OPR3 activation, which encode the key enzymes for JA synthesis. Nevertheless, JA defense gene such as PDF1.2 and JIP were not induced by NO in wt plants, but in NahG plant, suggesting that JA-production is under control of SA. This hypothesis could be confirmed by the determination of SA accumulation in wt plants, and JA accumulation in NahG plants but not in wt plants after NO treatment (**Figure 24**). SA

plays evidently a negative role in JA synthesis and induction of JA responsive defense genes. It is also consistent with the reported data that SA and related hydroxybenzonic acids inhibit wound response and wound-induced gene expression in tomato plants (Doherty et al., 1988; Pena-Cortés et al., 1993).

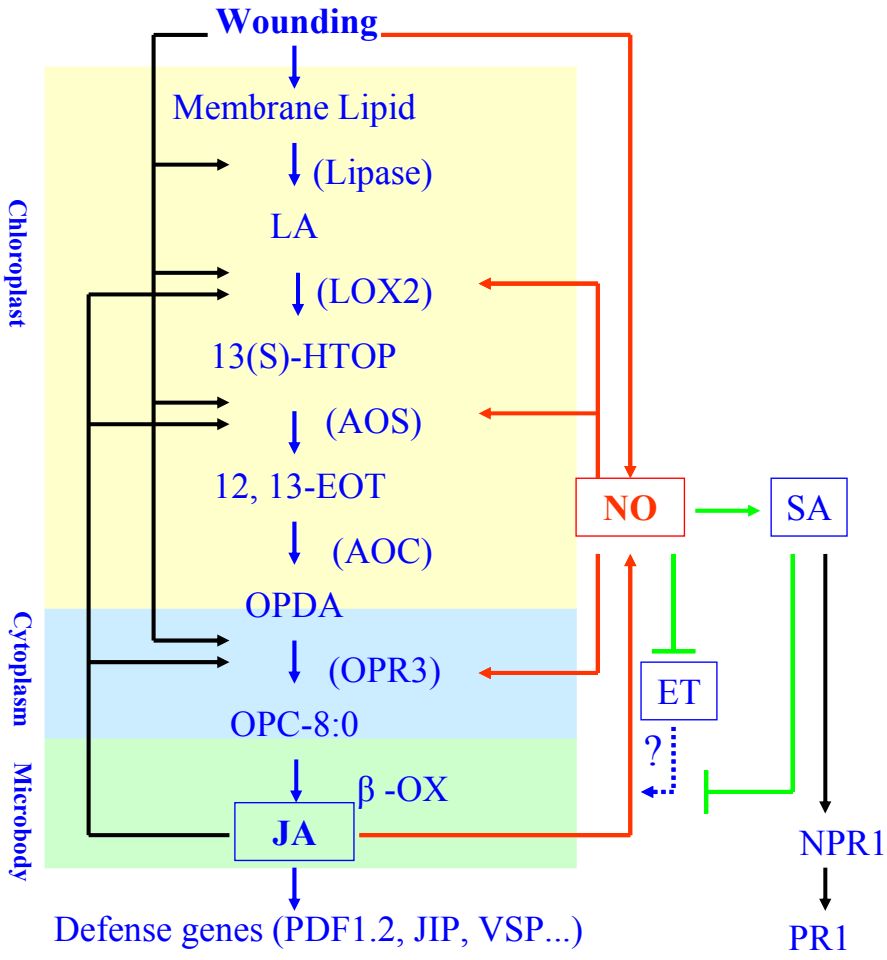


Figure 30: Summary of NO's effects on JA and SA signaling network. (Note: figure was drawn from the mold by Schaller, 2001). Black arrows means the reported results, red arrows indicate the new findings in this work, and green arrows show the findings in this work that are consistent with the reported. LA: α-linolenic acid; AOC: allene oxide cyclase; 13(S)-HPOT: (9Z, 11E, 15Z, 13S)-13-hydroperoxy-9,11,15-octadecatrienoic acid; 12, 13-EOT: (9Z, 11E, 15Z, 13S, 12R-12,13-epoxy-9,11,15-octadecatrienoic acid; OPDA: 12-oxo-10,15(Z)-octadecatrienoic acid; OPC-8:0 : 3-oxo-2(2'(Z)-pentenyl)-cyclopentane-1-octanoic acid; ET: Ethylene.

Considering that the antagonistic action between SA and JA signaling has been proposed to be central to the plant's ability to fine-tune the induction of plant defenses in response to different plants pest and pathogens (Kunkel and Brooks, 2002), NO seems to have dual effects on JA signaling pathway. On one hand, as Orozco-Cárdenas and Ryan suggested, NO is a negative regulator of wounding. This negative regulation is very possibly through SA signaling pathway (**Figures 23** and **24**). On the other hand, as data shown in this work (**Figures 19, 20** and **22**), NO induces JA synthesis machinery. NO seems to be a center factor of this fine-turning gene regulation, but how does NO exactly exert its effects is still less known. The discrepancy as observed in different works, e.g. SA accumulation by NO treatment was observed in tobacco by (Durner and Klessig, 1999) and in *Arabidopsis* (this work), but not in tobacco as reported by Orozco-Cárdenas and Ryan (2002), was very possible due to using different NO sources.

V. Conclusion

In the first stage of this work, protocols for fluorescent labeling cDNA microarray were set up and optimized. The process includes PCR amplification and purification of cDNA, slide fabrication (spotting and post process), probe cDNA synthesis and fluorescent labeling, hybridization and post washing, scanning, and data analysis. Using a cDNA microarray containing 330 stress genes, the transcripts of *Arabidopsis* suspension cells and plants treated with NO donor NOR3 and gaseous NO were analyzed, respectively. In response to NOR3 and gaseous NO, the transcript levels for many genes increased to maximum values 1 to 3h after treatment and then began to subside toward the baseline. This tendency was visible either on scatter plots or pseudocolor images. The array data have been proven to be reliable by northern blots. About 10 % of the tested genes were regulated by NO treatment and almost all of them are up-regulated. A lot of defense genes and anti-oxidant genes were up-regulated in suspension cells as well as in plants.

The most interesting gene induced by NO treatment in suspension cell is alternative oxidase (AOX1a). Only plants possess the alternative respiration pathway with AOX as a terminal electron acceptor. AOX has been suggested to be barely affected by NO. Data in this work show that NO affects cytochrome dependent respiration in *Arabidopsis thaliana*. Additionally, NO-treatment of *Arabidopsis* cell cultures strongly induced *AOX1a* transcription as determined cDNA microarray and Northern blot analyses. In accordance with transcript accumulation, NO treatment of suspension cells resulted in increased respiration through the alternative pathway. Addition of an AOX inhibitor to *Arabidopsis* cell cultures resulted in dramatically increased NO-sensitivity and cell death. In sum, these data suggest that NO induces the *AOX1a* gene and that AOX may participate to counteract the toxicity of NO.

Array data reveal that many genes related to JA signaling pathway were regulated by NO treatment *Arabidopsis thaliana* plants. Further investigation show, that NO interacts in a dual way with JA signaling. Real-time imaging by confocal laser scanning microscopy in conjunction with the NO-selective fluorescence indicator DAF-2 DA uncovered a strong NO burst after wounding or after treatment with jasmonic acid. Electron paramagnetic resonance (EPR) data confirmed this finding. The NO-burst was triggered within minutes, reminescent of the oxidative burst during hypersensitive responses. Expression profiling by cDNA microarray analyses of NO treated *Arabidopsis* leaves as well as Northern analyses revealed

that NO strongly induces key enzymes of JA biosynthesis such as allene oxide synthase and lipoxygenase. On the other hand, NO did not induce JA-responsive genes such as defensin. Gene expression analyses in the JA signaling mutant jar1 as well as in transgenic NahG plants, together with determination of signaling substances (JA, SA and ethylene) suggested that NO is a dual modulator of JA signaling. NO can act supportive through activation of biosynthetic enzymes, and retarding through accumulation of SA, an inhibitor of JA signaling. In sum, my data show that many genes are responsive to NO treatment, and they provide some ideas how NO might be integrated within the signaling pathways of plants.

VI. References

Adams, M. D., Kelley, J. M., Gocayne, J. D., Dubnick, M., Polymeropoulos, M. H., Xiao, H., Merril, C. R., Wu, A., Olde, B., Moreno, R. F., and et al. (1991). Complementary DNA sequencing: expressed sequence tags and human genome project. Science *252*, 1651-1656.

Alamillo, J. M., and García-Olmedo, F. (2001). Effects of urate, a natural inhibitor of peroxynitrite-mediated toxicity, in the response of *Arabidopsis thaliana* to the bacterial pathogen Pseudomonas syringae. Plant J *25*, 529-540.

Alvarez, M. E., Pennell, R. I., Meijer, P.-J., Ishikawa, A., Dixon, R. A., and Lamb, C. (1998). Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell *92*, 773-784.

Alwine, J. C., Kemp, D. J., and Stark, G. R. (1977). Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl-paper and hybridization with DNA probes. Proc Natl Acad Sci U S A *74*, 5350-5354.

Aslan, M., and Freeman, B. A. (2002). Oxidases and oxygenases in regulation of vascular nitric oxide signaling and inflammatory responses. Immunol Res *26*, 107-118.

Barchowsky, A., Klei, L. R., Dudek, E. J., Swartz, H. M., and James, P. E. (1999). Stimulation of reactive oxygen, but not reactive nirtogen species in vascular endothelial cells exposed to low levels of arsenite. Free Radic Biol Med *27*, 1405-1412.

Barroso, J. B., Corpas, F. J., Carreras, A., Sandalio, L. M., Valderrama, R., Palma, J. M., Lupianez, J. A., and del Rio, L. A. (1999). Localization of nitric-oxide synthase in plant peroxisomes. J Biol Chem *274*, 36729-36733.

Beers, E. P., and McDowell, J. M. (2001). Regulation and execution of programmed cell death in response to pathogens, stress and developmental cues. Curr Opin Plant Biol *4*, 561-567.

Beligni, M., and Lamattina, L. (1999a). Is nitric oxide toxic or protective? Trends Plant Sci *4*, 299-300.

Beligni, M. V., Fath, A., Bethke, P. C., Lamattina, L., and Jones, R. L. (2002). Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. Plant Physiol *129*, 1642-1650.

Beligni, M. V., and Lamattina, L. (1999b). Nitric oxide counteracts cytotoxic processes mediated by reactive oxygen species in plant tissues. Planta *208*, 337-344.

Beligni, M. V., and Lamattina, L. (2000). Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta *210*, 215-221.

Beligni, M. V., and Lamattina, L. (2001). Nitric oxide in plants: The history is just beginning. Plant Cell Environ *24*, 267-278.

Bellamy, T. C., Wood, J., Goodwin, D. A., and Garthwaite, J. (2000). Rapid desensitization of the nitric oxide receptor, soluble guanylyl cyclase, underlies diversity of cellular cGMP responses. Proc Natl Acad Sci U S A *97*, 2928-2933.

Bent, A. F., Innes, R. W., Ecker, J. R., and Staskawicz, B. J. (1992). Disease development in ethylene-insensitive *Arabidopsis thaliana* infected with virulent and avirulent Pseudomonas and Xanthomonas pathogens. Mol Plant Microbe Interact *5*, 372-378.

Berger, S. (2002). Jasmonate-related mutants of *Arabidopsis* as tools for studying stress signaling. Planta *214*, 497-504.

Berk, A. J., and Sharp, P. A. (1977). Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell *12*, 721-732.

Bird, A. (1992). The essentials of DNA methylation. Cell *70*, 5-8.

Bogdan, C. (2001a). Nitric oxide and the immune response. Nat Immunol *2*, 907-916.

Bogdan, C. (2001b). Nitric oxide and the regulation of gene expression. Trends Cell Biol *11*, 66-75.

Bolwell, G. P. (1999). Role of active oxygen species and NO in plant defence responses. Curr Opinion Plant Biol *2*, 287-294.

Brüne, B., Dimmler, S., Molina y Vedia, L., and Lapatina, E. G. (1994). Nitric oxide: a signal for ADP ribosylation of proteins. Life Sci *54*, 61-70.

Brüne, B., von Knethen, A., and Sandau, K. B. (1998). Nitric oxide and its role in apoptosis. Eur J Pharmacol *351*, 261-272.

Brunori, M., Giuffre, A., Sarti, P., Stubauer, G., and Wilson, M. T. (1999). Nitric oxide and cellular respiration. Cell Mol Life Sci *56*, 549-557.

Butt, Y. K., Lum, J. H., and Lo, S. C. (2003). Proteomic identification of plant proteins probed by mammalian nitric oxide synthase antibodies. Planta *216*, 762-771.

Cao, H., Glazebrook, J., Clarke, J. D., Volko, S., and Dong, X. (1997). The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell *88*, 57-63.

Caro, A., and Puntarulo, S. (1999). Nitric oxide generation by soybean embryonic axes. Possible effect of mitochondrial function. Free Red Res *31*, 205-212.

Chandok, M. R., Ytterberg, A. J., van Wijk, K. L., and Klessig, D. F. (2003). The pathogen-inducible nitric oxide synthase (iNOS) in plants is a variant of the P protein of the glycine decarboxylase complex. Cell *113*, 469-482.

Chang, C., Kwok, S. F., Bleecker, A. B., and Meyerowitz, E. M. (1993). *Arabidopsis* ethylene-response gene ETR1: similarity of product to two-component regulators. Science *262*, 539-544.

Chaudhuri, M., and Hill, G. C. (1996). Cloning, sequencing, and functional activity of Trypanosoma brucei brucei alternative oxidase. Mol Biochem Parasitol *83*, 125–129.

Chen, Y., Kamat, V., Dougherty, E. R., Bittner, M. L., Meltzer, P. S., and Trent, J. M. (2002). Ratio statistics of gene expression levels and applications to microarray data analysis. Bioinformatics *18*, 1207-1215.

Chen, Y. J., Kodell, R., Sistare, F., Thompson, K. L., Morris, S., and Chen, J. J. (2003). Normalization methods for analysis of microarray gene-expression data. J Biopharm Stat *13*, 57-74.

Cheong, Y. H., Chang, H.-S., Gupta, R., Wang, X., Zhu, T., and Luan, S. (2002). Transcriptional Profiling Reveals Novel Interactions between Wounding, Pathogen, Abiotic Stress, and Hormonal Responses in *Arabidopsis*. Plant Physiol *129*, 661–677.

Chivasa, S., and Carr, J. P. (1998). Cyanide restores *N* gene-mediated resistance to tobacco mosaic virus in transgenic tobacco expressing salicylic acid hydroxylase. Plant Cell *10*, 1489-1498.

Chivasa, S., Murphy, A. M., Naylor, M., and Carr, J. P. (1997). Salicylic acid interferes with tobacco mosaic virus replication via a novel salicylhydroxamic acid-sensitive mechanism. Plant Cell *9*, 547-557.

Clark, D., Durner, J., Navarre, D. A., and Klessig, D. F. (2000). Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. Mol Plant Microbe Interact *13*, 1380-1384.

Clarke, A., Desikan, R., Hurst, R. D., Hancock, J. T., and Neill, S. J. (2000). NO way back: nitric oxide and programmed cell death in *Arabidopsis thaliana* suspension cultures. Plant J *24*, 667-677.

Clarkson, A. B., Bienen, E. J., Pollakis, G., and Grady, R. W. (1989). Nitric oxide of blood stream forms of the parasite Trypanosoma brucei brucei is dependent on a plant-like alternative oxidase. J Biol Chem *264*, 17770–17776.

Clough, S. J., Fengler, K. A., Yu, I. C., Lippok, B., Smith, R. K., Jr., and Bent, A. F. (2000). The *Arabidopsis* dnd1 "defense, no death" gene encodes a mutated cyclic nucleotide-gated ion channel. Proc Natl Acad Sci U S A *97*, 9323-9328.

Cobbett, C., Goldsbrough, P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol *53*, 159-182.

Cohn, J., Sessa, G., and Martin, G. B. (2001). Innate immunity in plants. Curr Opin Immunol *13*, 55-62.

Cooney, R. V., Harwood, P. J., Custer, L. J., and Franke, A. A. (1994). Light-mediated conversion of nitrogen dioxide to nitric oxide by carotenoids. Environ Health Perspect *102*, 460-462.

Corpas, F. J., Barroso, J. B., and del Rio, L. A. (2001). Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. Trends Plant Sci *6*, 145-150.

Cueto, M., Hernandéz-Perera, O., Martin, R., Bentura, M. L., Rodrigo, J., Lamas, S., and Golvano, M. P. (1996). Presence of nitric oxide synthase activity in roots and nodules of *Lupinus albus*. FEBS Lett *398*, 159-164.

Dangl, J. L., and Jones, J. D. (2001). Plant pathogens and integrated defence responses to infection. Nature *411*, 826-833.

Delledonne, M., Xia, Y., Dixon, R. A., and Lamb, C. (1998). Nitric oxide functions as a signal in plant disease resistance. Nature *394*, 585-588.

Delledonne, M., Zeier, J., Marocco, A., and Lamb, C. (2001). Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci U S A *98*, 13454-13459.

Deora, A. A., Win, T., Vanhaesebroeck, B., and Lander, H. M. (1998). A redox-triggered ras-effector interaction. Recruitment of phosphatidylinositol 3'-kinase to Ras by redox stress. J Biol Chem *273*, 29923-29928.

Desikan, R., Griffiths, R., Hancock, J., and Neill, S. (2002). A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A *99*, 16314-16318.

Despres, C., DeLong, C., Glaze, S., Liu, E., and Fobert, P. R. (2000). The *Arabidopsis* NPR1/NIM1 protein enhances the DNA binding activity of a subgroup of the TGA family of bZIP transcription factors. Plant Cell *12*, 279-290.

Diehl, F., Grahlmann, S., Beier, M., and Hoheisel, J. D. (2001). Manufacturing DNA microarrays of high spot homogeneity and reduced background signal. Nucleic Acids Res *29*, E38.

Ding, H., and Demple, B. (2000). Direct nitric oxide signal transduction via nitrosylation of iron-sulfur centers in the SoxR transcription activator. Proc Natl Acad Sci U S A *97*, 5146-5150.

Dixon, D. P., Lapthorn, A., and Edwards, R. (2002). Plant glutathione transferases. Genome Biol *3*, Reviews3004.

Doares, S. H., Narvaez-Vasquez, J., Conconi, A., and Ryan, C. A. (1995). Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol *108*, 1741-1746.

Dobashi, K., Asayama, K., Nakane, T., Kodera, K., Hayashibe, H., and Nakazawa, S. (2001). Induction of glutathione peroxidase in response to inactivation by nitric oxide. Free Radic Res *35*, 319-327.

Doherty, H. M., Selvendran , R. R., and Bowles, D. J. (1988). The wound response of tomato plants can be inhibited by aspirin and related hydroxy-benzoic acids. Physiol Mol Plant Pathol *33*, 377–384.

Dong, X. (1998). SA, JA, ethylene, and disease resistance in plants. Curr Opin Plant Biol *1*, 316-323.

Durner, J., Gow, A. J., Stamler, J. S., and Glazebrook, J. (1999). Ancient origins of nitric oxide signaling in biological systems. Proc Natl Acad Sci U S A *96*, 14206-14207.

Durner, J., and Klessig, D. F. (1999). Nitric oxide as a signal in plants. Curr Opinion Plant Biol *2*, 369-372.

Durner, J., Wendehenne, D., and Klessig, D. F. (1998). Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci U S A *95*, 10328-10333.

Edwards, D. (2003). Non-linear normalization and background correction in one-channel cDNA microarray studies. Bioinformatics *19*, 825-833.

Eu, J. P., Liu, L., Zeng, M., and Stamler, J. S. (2000). An apoptotic model for nitrosative stress. Biochemistry *39*, 1040 -1047.

Ezaki, B., Katsuhara, M., Kawamura, M., and Matsumoto, H. (2001). Different mechanisms of four aluminum (Al)-resistant transgenes for Al toxicity in *Arabidopsis*. Plant Physiol *127*, 918-927.

Farmer, E. E., Weber, H., and Vollenweider, S. (1998). Fatty acid signaling in *Arabidopsis*. Planta *206*, 167-174.

Finkelstei, D., Ewing, R., Gollub, J., Sterky, F., Cherry, J. M., and Somerville, S. (2002). Microarray data quality analysis: lessons from the AFGC project. *Arabidopsis* Functional Genomics Consortium. Plant Mol Biol *48*, 119-131.

Flor, H. H. (1971). Current status of the gene-for-gene concept. Annu Rev Phytopathol *9*, 275-296.

Foissner, I., Wendehenne, D., Langebartels, C., and Durner, J. (2000). *In vivo* imaging of an elicitor-induced nitric oxide burst in tobacco. Plant J *23*, 817-824.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. (1993). Requirement of salicylic acid for the induction of systemic acquired resistance. Science *261*, 754-756.

Glazebrook, J. (2001). Genes controlling expression of defense responses in *Arabidopsis* - 2001 status. Curr Opin Plant Biol *4*, 301-308.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E. E., Crute, I. R., Holub, E. B., Hammerschmidt, R., and Ausubel, F. M. (1997). Phytoalexin-deficient mutants of *Arabidopsis* reveal that PAD4 encodes a regulatory factor and that four PAD genes contribute to downy mildew resistance. Genetics *146*, 381-392.

Gow, A. J., Luchsinger, B. P., Pawloski, J. R., Singel, D. J., and Stamler, J. S. (1999). The oxyhemoglobin reaction of nitric oxide. Proc Natl Acad Sci U S A *96*, 9027-9032.

Graziano, M., Beligni, M. V., and Lamattina, L. (2002). Nitric oxide improves internal iron availability in plants. Plant Physiol *130*, 1852-1859.

Gu, Y. Q., Wildermuth, M. C., Chakravarthy, S., Loh, Y. T., Yang, C., He, X., Han, Y., and Martin, G. B. (2002). Tomato transcription factors pti4, pti5, and pti6 activate defense responses when expressed in *Arabidopsis*. Plant Cell *14*, 817-831.

Hausladen, A., Privalle, C. T., Keng, T., DeAngelo, J., and Stamler, J. S. (1996). Nitrosative stress: activaton of the transcription factor OxyR. Cell *86*, 719-729.

Hegde, P., Qi, R., Abernathy, K., Gay, C., Dharap, S., Gaspard, R., Earle-Hughes, J., Snesrud, E., and Quackenbush, J. (2000). A Concise Guide to cDNA Microarray Analysis. Biotechniques *29*, 548-562.

Heidenreich, B., Mayer, K., Sandermann JR, H., and Ernst, D. (2001). Mercury-induced genes in *Arabidopsis thaliana*: identification of induced genes upon lon-term mercuric ion exposure. Plant Cell and Environment *24*, 1227-1234.

Hentze, M. W., and Kuhn, L. C. (1996). Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. Proc Natl Acad Sci U S A *93*, 8175-8182.

Hippeli, S., and Elstner, E. F. (1996). Mechanisms of oxygen activation during plant stress: Biochemical effects of air pollutants. J Plant Physiol *148*, 249-257.

Hmadcha, A., Bedoya, F. J., Sobrino, F., and Pintado, E. (1999). Methylation-dependent gene silencing induced by interleukin 1beta via nitric oxide production. J Exp Med *190*, 1595-1604.

Hoffman, T., Schmidt, J. S., Zheng, X. Y., and Bent, A. F. (1999). Isolation of ethylene-insensitive soybean mutants that are altered in pathogen susceptibility and gene-for-gene disease resistance. Plant Physiol *119*, 935-949.

Howard, C. J. (1980). Stratospheric chemistry and the ozone problem. In: Rodgers, M. and Powers E. (eds.), Oxygen and Oxy Radicals in Chemistry and Biology. Academic Press, New York, 535-589.

Huang, X., von Rad, U., and Durner, J. (2002). Nitric oxide induces the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension cells. Planta *215*, 914-923.

Jofuku, K. D., den Boer, B. G., Van Montagu, M., and Okamuro, J. K. (1994). Control of *Arabidopsis* flower and seed development by the homeotic gene APETALA2. Plant Cell *6*, 1211-1225.

Joseph-Horne, T., Hollomon, D. W., and Wood, P. M. (2001). Fungal respiration: a fusion of standard and alternative components. Biochim Biophys Acta *1504*, 179-195.

Kaiser, W. M., and Huber, S. C. (2001). Post-translational regulation of nitrate reductase: Mechanism, physiological relevance and environmental triggers. J Exp Bot *52*, 1981-1989.

Kaiser, W. M., Weiner, H., Kandlbinder, A., Tsai, C. B., Rockel, P., Sonoda, M., and Planchet, E. (2002). Modulation of nitrate reductase: some new insights, an unusual case and a potentially important side reaction. J Exp Bot *53*, 875-882.

Kalyanaraman, B. (1996). Detection of nitric oxide by electron spin resonance in chemical, photochemical, cellular, physiological, and pathophysiological systems. Methods Enzymol *268*, 168-187.

Kaptain, S., Downey, W. E., Tang, C., Philpott, C., Haile, D., Orloff, D. G., Harford, J. B., Rouault, T. A., and Klausner, R. D. (1991). A regulated RNA binding protein also possesses aconitase activity. Proc Natl Acad Sci U S A *88*, 10109-10113.

Kita, Y., Ozaki, R., Sakai, S., Sugimoto, T., Hirasawa, Y., Ohtsuka, M., Senoh, H., Yoshida, K., and Maeda, K. (1994). Antianginal effects of FK409, a new spontaneous NO releaser. Br J Pharmacol *113*, 1137-1140.

Klepper, L. (1991). NOx evolution by soybean leaves treated with salicylic acid and selected derivatives. Pest Biochem Physiol *39*, 43-48.

Kleschyov, A. L., Mollnau, H., Oelze, M., Meinertz, T., Huang, Y., Harrison, D. G., and Munzel, T. (2000). Spin trapping of vascular nitric oxide using colloid Fe(II)-diethyldithiocarbamate. Biochem Biophys Res Commun *275*, 672-677.

Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne, D., Kumar, D., Zhou, J. M., Shah, J., Zhang, S., Kachroo, P.*, et al.* (2000). Nitric oxide and salicylic acid signaling in plant defense. Proc Natl Acad Sci U S A *97*, 8849-8855.

Kojima, H., Nakatsubo, N., Kikuchi, K., Kawahara, S., Kirino, Y., Nagoshi, H., Hirata, Y., and Nagano, T. (1998). Detection and Imaging of nitric oxide with novel fluorescent indicators: Diaminofluoresceins. Anal Chem *70*, 2446-2453.

Kunkel, B. N., and Brooks, D. M. (2002). Cross talk between signaling pathways in pathogen defense. Curr Opin Plant Biol *5*, 325-331.

Laudert, D., Schaller, F., and Weiler, E. W. (2000). Transgenic Nicotiana tabacum and *Arabidopsis thaliana* plants overexpressing allene oxide synthase. Planta *211*, 163-165.

Lennon, A. M., Neuenschwander, U., Ribas-Carbo, M., Giles, L., Ryals, J. A., and Siedow, J. N. (1997). The effects of salicylic acid and tobacco mosaic virus infection on the alternative oxidase of tobacco. Plant Physiol *115*, 783-791.

León, J., Rojo, E., and Sanchez-Serrano, J. J. (2001). Wound signalling in plants. J Exp Bot *52*, 1-9.

Leshem, Y., and Haramaty, E. (1996). The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of Pisum sativum Linn. foliage. J Plant Physiol *148*, 258-263.

Leshem, Y. Y., Wills, R. B. H., and Ku, V. V. (1998). Evidence for the function of the free reduced gas – nitric oxide (NO·) as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol Biochem *36*, 825-826.

Liang, P., and Pardee, A. B. (1992). Differential Display of Eukaryotic Messenger-Rna By Means of the Polymerase Chain-Reaction. Science *257*, 967-971.

Lipton, S. A., Choi, Y. B., Pan, Z. H., Lei, S. Z., Chen, H. S., Sucher, N. J., Loscalzo, J., Singel, D. J., and Stamler, J. S. (1993). A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature *364*, 626-632.

Liu, L., Hausladen, A., Zeng, M., Que, L., Heitman, J., and Stamler, J. S. (2001). A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. Nature *410*, 490-494.

Lum, H. K., Butt, Y. K. C., and Lo, S. C. L. (2002). Hydrogen Peroxide Induces a Rapid Production of Nitric Oxide in Mung Bean (Phaseolus aureus). Nitric Oxide *6*, 205-213.

Lund, S. T., Stall, R. E., and Klee, H. J. (1998). Ethylene regulates the susceptible response to pathogen infection in tomato. Plant Cell *10*, 371-382.

Mata, C. G., and Lamattina, L. (2001). Nitric Oxide Induces Stomatal Closure and Enhances the Adaptive Plant Responses against Drought Stress. Plant Physiol *126*, 1196-1204.

Mathieu, C., Moreau, S., Frendo, P., Puppo, A., and Davies, M. J. (1998). Direct detection of radicals in intact soybean nodules: presence of nitric oxide-leghemoglobin complexes. Free Radic Biol Med *24*, 1242-1249.

Matthews, J. R., Botting, C. H., Panico, M., Morris, H. R., and Hay, R. T. (1996). Inhibition of NF-kappaB DNA binding by nitric oxide. Nucleic Acids Res *24*, 2236-2242.

Maxwell, D. P., Wang, Y., and McIntosh, L. (1999). The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci U S A *96*, 8271-8276.

Mayer, B., and Hemmens, B. (1997). Biosynthesis and action of nitric oxide in mammalian cells. Trends Biochem Sci *22*, 477-481.

McDowell, J. M., and Dangl, J. L. (2000). Signal transduction in the plant immune response. Trends Biochem Sci *25*, 79-82.

Meeuse, B. J. D. (1975). Thermogenic respiration in aroids. Annu Rev Plant Physiol *26*, 117-126.

Meuwly, P., and Metraux, J. P. (1993). Ortho-anisic acid as internal standard for the simultaneous quantitation of salicylic acid and its putative biosynthetic precursors in cucumber leaves. Anal Biochem *214*, 500-505.

Millar, A. H., and Day, D. A. (1996). Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. FEBS Lett *398*, 155-158.

Millar, A. H., and Day, D. A. (1997). Alternative solutions to radical problems. Trends Plant Sci *2*, 289-290.

Mueller, M. J., Brodschelm, W., Spannagl, E., and Zenk, M. H. (1993). Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. Proc Natl Acad Sci U S A *90*, 7490-7494.

Murray, A. J. S., and Wellburn, A. R. (1985). Differences in nitrogen metabolism between cultivars of tomato and pepper during exposure to glasshouse atmospheres containing oxides of nitrogen. Environ Pollution *39*, 303-316.

Nappi, A. J., Vass, E., Frey, F., and Carton, Y. (2000). Nitric oxide involvement in Drosophila immunity. Nitric Oxide *4*, 423-430.

Nathan, C. (1992). Nitric oxide as a secretory product of mammalian cells. Faseb J *6*, 3051-3064.

Nathan, C., and Shiloh, M. U. (2000). Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Natl Acad Sci U S A *97*, 8841-8848.

Nathan, C., and Xie, Q.-W. (1994). Nitric oxide synthases: Roles, tolls, and controls. Cell *78*, 915-918.

Navarre, D. A., Wendehenne, D., Durner, J., Noad, R., and Klessig, D. F. (2000). Nitric oxide modulates the activity of two tobacco enzymes aconitase. Plant Physiol *122*, 573-582.

Neill, S. J., Desikan, R., Clarke, A., and Hancock, J. T. (2002a). Nitric oxide is a novel component of abscisic Acid signaling in stomatal guard cells. Plant Physiol *128*, 13-16.

Neill, S. J., Desikan, R., Clarke, A., Hurst, R. D., and Hancock, J. T. (2002b). Hydrogen peroxide and nitric oxide as signalling molecules in plants. J Exp Bot *53*, 1237-1247.

Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N., and Ohashi, Y. (1998). Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol *39*, 500–507.

Nishimura, I., Hayamizu, T., and Yanagisawa, Y. (1986). Reduction of NO2 to NO by rush and other plants. Environ Sci Technol *20*, 413-416.

Norman-Setterblad, C., Vidal, S., and Palva, E. T. (2000). Interacting signal pathways control defense gene expression in *Arabidopsis* in response to cell wall-degrading enzymes from Erwinia carotovora. Mol Plant Microbe Interact *13*, 430-438.

Okubo, K., Hori, N., Matoba, R., Niiyama, T., Fukushima, A., Kojima, Y., and Matsubara, K. (1992). Large scale cDNA sequencing for analysis of quantitative and qualitative aspects of gene expression. Nat Genet *2*, 173-179.

Orozco-Cárdenas, M., Nárvaez-Vásquez, J., and Ryan, C. (2001). Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell *13*, 179-191.

Orozco-Cárdenas, M., and Ryan, C. A. (1999). Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. Proc Natl Acad Sci U S A *96*, 6553-6557.

Orozco-Cárdenas, M. L., and Ryan, C. (2002). Nitric oxide negatively modulates wound signaling in tomato plants. Plant Physiol *130*, 487-493.

Pagnussat, G. C., Simontacchi, M., Puntarulo, S., and Lamattina, L. (2002). Nitric oxide is required for root organogenesis. Plant Physiol *129*, 954-956.

Palmer, R. M., Ferrige, A. G., and Moncada, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature *327*, 524-526.

Park, J. H., Halitschke, R., Kim, H. B., Baldwin, I. T., Feldmann, K. A., and Feyereisen, R. (2002). A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. Plant J *31*, 1-12.

Pedroso, M. C., Magalhaes, J. R., and Durzan, D. (2000). A nitric oxide burst precedes apoptosis in angiosperm and gymnosperm callus cells and foliar tissues. J Exp Bot *51*, 1027-1036.

Pena-Cortés, H., Albrecht, T., Prat, S., Weiler, E. W., and Willmitzer, L. (1993). Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. Planta *191*, 123-138.

Pieterse, C. M. J., and Van Loon, L. C. (1999). Salicylic acid-independent plant defence pathways. Trends Plant Sci *4*, 52-58.

Quesada, A., Goméz-García, I., and Fernandéz, E. (2000). Involvement of chloroplast and mitochondria redox valves in nitrate assimilation. Trends Plant Sci *5*, 463-464.

Ramdas, L., Coombes, K. R., Baggerly, K., Abruzzo, L., Highsmith, W. E., Krogmann, T., Hamilton, S. R., and Zhang, W. (2001). Sources of nonlinearity in cDNA microarray expression measurements. Genome Biol *2*, RESEARCH0047.

Raskin, I., Ehmann, A., Melander, W. R., and Meeuse, B. J. D. (1987). Salicylic acid - a natural inducer of heat production in Arum lilies. Science *237*, 1601-1602.

Reiter, C. D., Teng, R. J., and Beckman, J. S. (2000). Superoxide reacts with nitric oxide to nitrate tyrosine at physiological pH via peroxynitrite. J Biol Chem *275*, 32460-32466.

Reymond, P., and Farmer, E. E. (1998). Jasmonate and salicylate as global signals for defense gene expression. Curr Opin Plant Biol *1*, 404-411.

Reymond, P., Weber, H., Damond, M., and Farmer, E. E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell *12*, 707-720.

Rhoads, D. M., and McIntosh, L. (1992). Salicylic acid regulation of respiration in higher plants: alternative oxidase expression. Plant Cell *4*, 1131-1139.

Rhoads, D. M., and McIntosh, L. (1993). Cytochrome and alternative pathway respiration in tobacco; effects of salicylic acid. Plant Physiol *103*, 877-883.

Ribeiro, E. A., Cunha, F. Q., Tamashiro, W. M. S. C., and Martins, I. S. (1999). Growth phase-dependent subcellular localization of nitric oxide synthase in maize cells. FEBS Lett *445*, 283-286.

Richards, K. D., Schott, E. J., Sharma, Y. K., Davis, K. R., and Gardner, R. C. (1998). Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. Plant Physiol *116*, 409-418.

Rockel, P., Strube, F., Rockel, A., Wildt, J., and Kaiser, W. M. (2002). Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. J Exp Bot *53*, 103-110.

Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H.-Y., and Hunt, M. D. (1996). Systemic acquired resistance. Plant Cell *8*, 1809-1819.

Ryan, C. A. (2000). The systemin signaling pathway: differential activation of plant defensive genes. Biochim Biophys Acta *1477*, 112-121.

Saffert, A., Hartmann-Schreier, J., Schon, A., and Schreier, P. (2000). A dual function alpha-dioxygenase-peroxidase and NAD(+) oxidoreductase active enzyme from germinating pea rationalizing alpha-oxidation of fatty acids in plants. Plant Physiol *123*, 1545-1552.

Saisho, D., Nambara, E., Naito, S., Tsutsumi, N., Hirai, A., and Nakazono, M. (1997). Characterization of the gene family for alternative oxidase from *Arabidopsis thaliana*. Plant Mol Biol *35*, 585-596.

Sandermann, H. (2001) Ozon Entstehung, Wirkung, Risiken. Verlag C. H. Beck oHG, München, 23-28.

Schaller, F. (2001). Enzymes of the biosynthesis of octadecanoid-derived signalling molecules. J Exp Bot *52*, 11-23.

Schena, M., Shalon, D., Davis, R. W., and Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science *270*, 467-470.

Schena, M., Shalon, D., Heller, R., Chai, A., Brown, P. O., and Davis, R. W. (1996). Parallel human genome analysis: microarray-based expression monitoring of 1000 genes. Proc Natl Acad Sci U S A *93*, 10614-10619.

Schmidt, H. H., and Walter, U. (1994). NO at work. Cell *78*, 919-925.

Schuler, G. D. (1997). Pieces of the puzzle: expressed sequence tags and the catalog of human genes. J Mol Med *75*, 694-698.

Shah, J., Tsui, F., and Klessig, D. F. (1997). Characterization of a Salicylic Acid-insensitive mutant (sai1) of *Arabidopsis thaliana*, identified in a selective screen utilizing the SA-Inducible expression of the tms2 gene. Mol Plant Microbe Interact *10*, 69-78.

Shalon, D., Smith, S. J., and Brown, P. O. (1996). A DNA microarray system for analyzing complex DNA samples using two-color fluorescent probe hybridization. Genome Res *6*, 639-645.

Shinyashiki, M., Chiang, K. T., Switzer, C. H., Gralla, E. B., Valentine, J. S., Thiele, D. J., and Fukuto, J. M. (2000). The interaction of nitric oxide (NO) with the yeast transcription factor Ace1: A model system for NO-protein thiol interactions with implications to metal metabolism. Proc Natl Acad Sci U S A *97*, 2491-2496.

Shirasu, K., and Schulze-Lefert, P. (2000). Regulators of cell death in disease resistance. Plant Mol Biol *44*, 371-385.

Shiva, S., Brookes, P. S., Patel, R. P., Anderson, P. G., and Darley-Usmar, V. M. (2001). Nitric oxide partitioning into mitochondrial membranes and the control of respiration at cytochrome c oxidase. Proc Natl Acad Sci U S A *98*, 7212-7217.

Simon-Plas, F., Elmayan, T., and Blein, J.-P. (2002). The plasma membrane oxidase NtrbohD is responsible for AOS production in elicited tobacco cells. Plant J *31*, 137-148.

Simons, B. H., Millenaar, F. F., Mulder, L., Van Loon, L. C., and Lambers, H. (1999). Enhanced expression and activation of the alternative oxidase during infection of *Arabidopsis* with Pseudomonas syringae pv tomato. Plant Physiol *120*, 529-538.

Spiecker, M., Peng, H. B., and Liao, J. K. (1997). Inhibition of endothelial vascular cell adhesion molecule-1 expression by nitric oxide involves the induction and nuclear translocation of IkappaBalpha. J Biol Chem *272*, 30969-30974.

Stamler, J. S. (1994). Redox signaling: nitrosylation and related target interactions of nitric oxide. Cell *78*, 931-936.

Stamler, J. S., and Hausladen, A. (1998). Oxidative modifications in nitrosative stress. Nat Struct Biol *5*, 247-249.

Stamler, J. S., Singel, D. L., and Loscalzo, J. (1992). Biochemistry of nitric oxide and its redox-activated forms. Science *258*, 18981902.

Stancovski, I., and Baltimore, D. (1997). NF-kappaB activation: the I kappaB kinase revealed? Cell *91*, 299-302.

Sticher, L., Mauch-Mani, B., and Metreaux, J. P. (1997). Systemic acquired resistance. Annu Rev Phytopathol *35*, 235-270.

Stintzi, A., and Browse, J. (2000). The *Arabidopsis* male-sterile mutant, opr3, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc Natl Acad Sci U S A *97*, 10625-10630.

Stöhr, C., Strube, F., Marx, G., Ullrich, W. R., and Rockel, P. (2001). A plasma membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from nitrite. Planta *212*, 835-841.

Stuehr, D. J. (1999). Mammalian nitric oxide synthases. Biochim Biophys Acta *1411*, 217-230.

Suzuki, N., Kojima, H., Urano, Y., Kikuchi, K., Hirata, Y., and Nagano, T. (2002). Orthogonality of calcium concentration and ability of 4,5- diaminofluorescein to detect NO. J Biol Chem *277*, 47-49.

Tatoyan, A., and Giulivi, C. (1998). Purification and characterization of a nitric-oxide synthase from rat liver mitochondria. J Biol Chem *273*, 11044-11048.

Thimm, O., Essigmann, B., Kloska, S., Altmann, T., and Buckhout, T. J. (2001). Response of *Arabidopsis* to iron deficiency stress as revealed by microarray analysis. Plant Physiol *127*, 1030-1043.

Thomma, B., Eggermont, K., Tierens, K., and Broekaert, W. F. (1999). Requirement of functional ethylene-insensitive 2 gene for efficient resistance of *Arabidopsis* to infection by Botrytis cinerea. Plant Physiol *121*, 1093-1101.

Tsuchiya, K., Takasugi, M., Minakuchi, K., and Fukuzawa, K. (1996). Sensitive quantitation of nitric oxide by EPR spectroscopy. Free Radic Biol Med *21*, 733-737.

Tsuji, H., Nakazono, M., Saisho, D., Tsutsumi, N., and Hirai, A. (2000). Transcript levels of the nuclear-encoded respiratory genes in rice decrease by oxygen deprivation: evidence for involvement of calcium in expression of the alternative oxidase 1a gene. FEBS Lett *471*, 201-204.

Tuomainen, J., Betz, C., Kangasjarvi, J., Ernst, D., Yin, Z. H., Langebartels, C., and Sandermann, H. (1997). Ozone induction of ethylene emission in tomato plants: regulation by differential accumulation of transcripts for the biosynthetic enzymes. Plant J *12*, 1151-1162.

Umansky, V., Hehner, S. P., Dumont, A., Hofmann, T. G., Schirrmacher, V., Droge, W., and Schmitz, M. L. (1998). Co-stimulatory effect of nitric oxide on endothelial NF-kappaB implies a physiological self-amplifying mechanism. Eur J Immunol *28*, 2276-2282.

Van Breusegem, F., Vranová, E., Dat, J. F., and Inzé, D. (2001). The role of active oxygen species in plant signal transduction. Plant Sci *161*, 405-414.

Van Camp, W., Van Montagu, M., and Inzé, D. (1998). H_2O_2 and NO: redox signals in disease resistance. Trends Plant Sci *3*, 330-334.

Van Loon, L. C. (1999). Occurrence and properties of plant pathogenesis-related proteins. In Pathogenesis-related proteins in plants., S. K. Datta, and S. Muthukrishnan, eds. (Boca Raton, CRC Press), pp. 1-19.

Van Loon, L. C., Bakker, P. A. H. M., and Pieterse, C. M. J. (1998). Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol *36*, 453-483.

Vanlerberghe, G. C., and McIntosh, L. (1997). Alternative Oxidase: From Gene to Function. Annu Rev Plant Physiol Plant Mol Biol *48*, 703-734.

Velculescu, V. E., Zhang, L., Vogelstein, B., and Kinzler, K. W. (1995). Serial analysis of gene expression. Science *270*, 484-487.

Verma, I. M., Stevenson, J. K., Schwarz, E. M., Van Antwerp, D., and Miyamoto, S. (1995). Rel/NF-kappa B/I kappa B family: intimate tales of association and dissociation. Genes Dev *9*, 2723-2735.

Vodovotz, Y., Chesler, L., Chong, H., Kim, S. J., Simpson, J. T., DeGraff, W., Cox, G. W., Roberts, A. B., Wink, D. A., and Barcellos-Hoff, M. H. (1999). Regulation of transforming growth factor beta1 by nitric oxide. Cancer Res *59*, 2142-2149.

Wang, C., Zien, C. A., Afitlhile, M., Welti, R., Hildebrand, D. F., and Wang, X. (2002). Involvement of Phospholipase D in Wound-Induced Accumulation of Jasmonic Acid in *Arabidopsis*. Plant Cell *12*, 2237-2246.

Wang, R., Guegler, K., LaBrie, S. T., and Crawford, N. M. (2000). Genomic analysis of a nutrient response in *Arabidopsis* reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. Plant Cell *12*, 1491-1509.

Wellburn, A. R. (1990). Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers? New Phytol *115*, 395-429.

Wendehenne, D., Pugin, A., Klessig, D. F., and Durner, J. (2001). Nitric oxide: comparative synthesis and signaling in animal and plant cells. Trends Plant Sci *6*, 177-183.

Wilson, D. L., Buckley, M. J., Helliwell, C. A., and Wilson, I. W. (2003). New normalization methods for cDNA microarray data. Bioinformatics *19*, 1325-1332.

Wojtaszek, P. (2000). Nitric oxide in plants. To NO or not to NO. Phytochemistry *54*, 1-4.

Xu, Y., Chang, P., Liu, D., Narasimhan, M., Raghothama, K., Hasegawa, P., and Bressan, R. (1994). Plant defense genes are synergistically induced by ethylene and methyl jasmonate. Plant Cell *6*, 1077-1085.

Yamamoto, T., Yuyama, K., Nakamura, K., Kato, T., and Yamamoto, H. (2000). Kinetic characterization of the nitric oxide toxicity for PC12 cells: effect of half-life time of NO release. Eur J Pharmacol *397*, 25-33.

Yamasaki, H. (2000). Nitrite-dependent nitric oxide production pathway: implications for involvement of active nitrogen species in photoinhibition in vivo. Philos Trans R Soc Lond B Biol Sci *355*, 1477-1488.

Yamasaki, H., and Sakihama, Y. (2000). Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. FEBS Lett *468*, 89-92.

Yamasaki, H., Sakihama, Y., and Takahashi, A. (1999). An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trends Plant Sci *4*, 128-129.

Yamasaki, H., Shimoji, H., Ohshiro, Y., and Sakihama, Y. (2001). Inhibitory effects of nitric oxide on oxidative phosphorylation in plant mitochondria. Nitric Oxide *5*, 261-270.

Yip, J. Y., and Vanlerberghe, G. C. (2001). Mitochondrial alternative oxidase acts to dampen the generation of active oxygen species during a period of rapid respiration induced to support a high rate of nutrient uptake. Physiol Plant *112*, 327-333.

Yu, I. C., Parker, J., and Bent, A. F. (1998). Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis* dnd1 mutant. Proc Natl Acad Sci U S A *95*, 7819-7824.

Zhao, Y., Brandish, P. E., Ballou, D. P., and Marletta, M. A. (1999). A molecular basis for nitric oxide sensing by soluble guanylate cyclase. Proc Natl Acad Sci U S A *96*, 14753-14758.

Zielinski, R. E. (1998). Calmodulin and calmodulin-binding proteins in plants. Annu Rev Plant Physiol Plant Mol Biol *49*, 697–725.

VII. Supplement

Tabele S1. The gene that used for microarray

Function	Gene#	Position	EST#
Small nuclear ribonucleoprotein U1A	At2g47580	D7A01	38C6T7
Rubisco activase	At2g39730	D7A02	63A8T7
RuBisCO small subunit 1b	At5g38430	D7A03	75B10T7
Ubiquitin-conjucating enzyme E2- 17kD	At5g41700	D7A04	115N7T7
Ribulose bisphosphate carboxylase	At5g38410	D7A05	116H8T7
ATP synthase beta chain; mitochondrial precursor	At5g08670	D7A06	116L17T7
Putative triosephosphate isomerase	At2g21170	D7A07	157K7T7
Glycolate oxidase- like protein	At4g18360	D7A08	160K18T7
23 kDa polypeptide of oxygen-evolving comlex	At1g06680	D7A09	175C3T7
Chloroplast triose phosphate translocator precursor	At5g38420	D7A10	184D20T7
Rubisco small chain 1A precursor	At1g67090	D7A11	210I21T7
Polyubiquitin (ubq3)	At5g03240	D7A12	227N6T7
Lycopene beta-cyclase	At3g10230	D7B01	TC161843
Lycopene epsilon-cyclase	At5g57030	D7B02	TC149909
Carotene beta-hydroxylase	At5g52570	D7B03	TC154545
Zeaxanthin epoxidase	At5g67030	D7B04	TC150621
Violaxanthin de-epoxidase	At1g08550	D7B05	TC162934
neoxanthin cleavage protein		D7B06	
AtCLH2	At5g43860	D7B07	TC156459
Ap2 unknown protein	At4g37000	D7B08	TC164245
Ferulic acid 5-hydroxylase	At4g36220	D7B09	TC150466
Lipase-like protein(PAD4)	At3g52430	D7B11	AF188329
Defense; no death (DND1)	At5g15410	D7B12	AF280939
CYP90C1	At4g36380	D7C01	TC104445
NDK nucleoside diphosphate kinase type 1	At4g09320	D7C02	TC109414
Family II lipase EXL4	At1g75910	D7C03	TC121458
Strong similarity to glycoprotein EP1	At1g78820	D7C04	TC103556
Glutathione transferase(ERD13)	At2g30870	D7C05	TC115791
Axi 1-like protein	At2g44500	D7C06	TC121990
Salt stress inducible sGTP binding prot. Ran1 homolog	At5g55190	D7C07	TC115749
Aldose 1-epimerase-like protein	At3g47800	D7C08	TC104379

Calmodulin-3	At3g56800	D7C09	TC115610
Putative dicarboxylate diiron protein (Crd1)	At3g56940	D7C10	TC115432
Aquaporin (plasma membrane intrinsic protein 1B)	At2g45960	D7C11	TC121277
Plastocyanin precursor	At1g76100	D7C12	TC115945
Putative nematode-resistance protein	At2g40000	D7D01	TC121546
Step II splicing factor	At1g65660	D7D02	TC123026
Nitrilase 1	At3g44310	D7D03	TC109542
Dehydration-induced protein (ERD15)	At2g41430	D7D04	TC103567
Hypothetical protein	At3g47833	D7D05	TC113523
Salt-tolerance zinc finger protein	At1g27730	D7D06	TC121530
Putative CCCH-type zinc finger protein	At2g19810	D7D07	TC105445
Defense-related protein	At4g30530	D7D08	TC109709
Pyridoxine biosynthesis protein - like	At5g01410	D7D09	TC103559
Inorganic pyrophosphatase - like protein	At5g09650	D7D10	TC104037
Transmembrane protein TMP-B	At1g01620	D7D11	TC115452
14-3-3-Like protein GF14 omega	At1g78300	D7D12	TC103541
Pyruvate dehydrogenase E1 alpha subunit	At1g24180	D7E01	TC104043
Nucleoside diphosphate kinase Ia	At5g63310	D7E02	TC121793
Cyclic nucleotide and calmodulin-regulated ion channel	At5g57940	D7E03	TC113425
Glutamate decarboxylase	At3g17760	D7E04	TC105852
Cysteine proteinase	At4g16190	D7E05	TC103557
Cystathionine gamma-synthase	At3g01120	D7E06	TC109480
GTP-binding protein-like	At5g47520	D7E07	TC118651
Violaxanthin de-epoxidase precursor	At1g08550	D7E08	TC109750
Uncoupling protein (ucp/PUMP)	At3g54110	D7E09	TC115635
ARR1 protein	At3g16857	D7E10	TC122029
Putative NADPH dep. mannose 6-phosphate reductase	At2g21250	D7E11	AC006841
Cysteine proteinase inhibitor	At5g05110	D7E12	TC112427
Arginine decarboxylase SPE2	At4g34710	D7F01	TC103568
Cold-regulated protein kin2	At5g15970	D7F02	TC121375
Adenylate kinase	At5g50370	D7F03	TC104860
Casein kinase II alpha chain 2	At3g50000	D7F04	TC116352
Hypothetical protein	At1g32370	D7F05	NP222441
Arginine-tRNA-protein transferase 1 homolog	At5g05700	D7F06	TC117679
Peroxidase prxr1	At4g21960	D7F07	TC121255
Putative endochitinase	At1g05850	D7F08	TC121326
Aldehyde dehydrogenase (NAD+)-like protein	At3g48000	D7F09	TC109707
Cellulose synthase catalytic subunit	At5g05170	D7F10	TC103593

Glutathione S-transferase	At2g30860	D7F11	TC109437
GT-1	At1g13450	D7F12	TC117394
Calcium-dependent protein kinase(CDPK6)	At4g23650	D7G01	TC121315
Alanine aminotransferase	At1g23310	D7G02	TC109442
Senescence-associated protein sen1	At4g35770	D7G03	TC103526
Formate dehydrogenase; mitocho	At5g14780	D7G04	TC121377
26S proteasome AAA-ATPase subunit RPT6a	At5g19990	D7G05	TC103786
T14P8 unknown protein	At4g02340	D7G06	TC103878
Zinc finger protein 4;	At1g66140	D7G07	TC110518
Hypothetical protein; putative Tub family protein	At2g18280	D7G08	TC115587
Beta-galactosidase	At3g13750	P0A01	4G4T7P
Small nuclear ribonucleoprotein U1A	At2g47580	P0A02	38C6T7
Ribulose bisphosphate carboxylase/oxygenase activase	At2g39730	P0A03	63A8T7
Rubisco small chain 1B (precursor)	At5g38430	P0A04	75B10T7
Expansin At-EXP5	At3g29030	P0A05	82C2T7
Tropinone reductase (putative)	At2g29330	P0A06	82C4T7
DNA repair protein RAD23 homolog	At5g38470	P0A07	82G7T7
DNA-binding protein GT-2- A. thaliana	At1g76890	P0A08	87B3T7
Nucleolar protein-like	At5g55920	P0A09	96K22T7
Mac9 unknown protein	At5g61820	P0A10	98A9T7
Ubiquitin-conjucating enzyme	At1g64230	P0A11	115N7T7
Ribulose bisphosphate carboxylase	At5g38410	P0A12	116H8T7
H+-transporting ATP synthase beta chain	At5g08670	P0B01	116L17T7
Cytochrome P450	At2g14100	P0B02	118B4T7
Receptor protein kinase -like protein	At5g49660	P0B03	123P20T7
Serine/threonine kinase- like protein	At4g23220	P0B04	126O20T7
Sulfate transporter	At1g77990	P0B05	130L5T7
Ripening-induced protein and major latex protein	At1g23130	P0B06	142N21T7
Glycine-rich RNA-binding protein 7	At2g21660	P0B07	144G22T7
Fasciclin-like arabinogalactan-protein 7	At2g04780	P0B08	156K12T7
Triose phosphate isomerase chloro. precursor	At2g21170	P0B09	157K7T7
N-Acetylglucosamine transferase	At1g73740	P0B10	160H21T7
Glycolate oxidase- like protein	At4g18360	P0B11	160K18T7
Eukaryotic initiation factor 4A-2	At1g54270	P0B12	165F20T7
Fructose bisphosphate aldolase; cytoplasmic isozyme	At2g36460	P0C01	166L1T7
Pyruvate dehydrogenase E1 alpha subunit	At1g24180	P0C02	169E9T7
Putative glucosyltransferase	At1g73880	P0C03	173O14T7
MADS box transcription factors (similar to)	At5g65050	P0C04	174K6T7

Nonspecific lipid-transfer protein 3 precursor (LTP3)	At3g08770	P0C05	174N16T7
MQK4 putative protein	At5g16470	P0C06	175A20T7
23 kDa polypeptide of oxygen-evolving comlex	At1g06680	P0C07	175C3T7
Chalcone isomerase	At3g55120	P0C08	177A20T7
F12P19 putative protein	At1g65980	P0C09	177H3T7
Calreticulin (AtCRTL)	At1g09210	P0C10	177N21T7
Microbody NAD-dependent malate dehydrogenase	At5g09660	P0C11	178D7T7
DAG-like protein; chloroplast precursor	At3g15000	P0C12	182C14T7
Phosphoglycerate kinase precursor	At1g79550	P0D01	183F12T7
Heat shock transcription factor-like protein	At5g45710	P0D02	183O20T7
Proline-rich unknown protein	At2g45180	P0D03	184C24T7
Chloroplast triose phosphate translocator precursor	At5g46110	P0D04	184D20T7
20S proteasome beta subunit PBB2	At5g40580	P0D05	185N21T7
Drought-induced protein Dr4 - A. thaliana	At1g73330	P0D06	187H10T7
Ccr4-associated factor homolog (putative)	At5g10960	P0D07	187N5T7
Putative YME1 ATP-dependant protease	At2g21660	P0D08	192K14T7
Histone H2A - like protein	At5g54640	P0D09	193G7T7
4-Coumarate-CoA ligase-like protein	At3g48990	P0D10	196I16T7
Ribosomal protein L13a	At5g48760	P0D11	197G2T7
Xyloglucan endotransglycolase-related protein XTR-7	At4g14130	P0D12	197L23T7
Heat shock cognate 70kD protein	At3g09440	P0E01	197P7T7
Transcription factor II homolog	At4g31720	P0E02	199J4T7
Ornithine carbamoyltransferase	At1g75330	P0E03	199P13T7
Wu_e_35 putative protein	At5g20700	P0E04	203O23T7
Rubisco small subunit	At1g67090	P0E05	210I21T7
GF14 epsilon isoform; 14-3-3 protein GF14 epsilon;	At1g22300	P0E06	214C12T7
Putative endochitinase	At1g05850	P0E07	219H22T7
Elongation factor 1-beta A1 (EF-beta)	At2g18110	P0E08	220N9T7
Seed imbibition protein	At5g20250	P0E09	222A14T7
Auxin-responsive protein IAA10	At1g04100	P0E10	222G9T7
Polyubiquitin; polyubiquitin (ubq3)	At5g03240	P0E11	227N6T7
AP2 domain containing protein RAP2.5	At3g15210	P0E12	227N9T7
Copper homeostasis factor	At3g56240	P1A01	31B12T7
RD29A; low-temperature-induced protein 78	At5g52310	P1A02	31G2T7
Antifungal protein like(PDF1.2)	At5g44420	P1A03	37F10T7
Serine/threonine kinase-like protein	At4g23300	P1A04	90J22T7
Ferrodoxin NADP oxidoreductase - like protein	At1g30510	P1A05	93E3T7
Photosystem II type I chlorophyll a/b binding	At2g34430	P1A06	119M8T7

protein			
EAT1 ethylene forming enzyme	At1g05010	P1A07	121E19T7
unknown protein		P1A08	127L23T7
Acyl-CoA independent ceramide synthase	At4g22330	P1A09	131K21T7
Ethylene response sensor	At2g40940	P1A10	144E10T7
F5O11.2	At1g12270	P1A11	169G6T7
ORF1; CXc750	At1g31580	P1A12	173E15T7
Glutathione S-transferase	At4g02520	P1B01	175C20T7
Chalcome synthase	At5g13930	P1B02	177N23T7
Photosystem II type I chlorophyll a/b binding protein	At2g34420	P1B03	187D7T7
Nitrate reductase	At1g77760	P1B04	187O3T7
Beta-1; 3-glucanase-like protein	At3g55430	P1B05	191A8T7
L-ascorbate peroxidase	At1g07890	P1B06	193B8T7
Alternative oxidase 1a	At3g22370	P1B07	214L16T7
Alpha-DOX1 fatty acid dioxygenase	At3g01420	P1B08	218B16T7
Blue copper binding protein	At5g20230	P1B09	225I19T7
Peroxidase	At3g49120	P1B10	281H9T7
Basic chitinase	At3g12500	P1B11	92G1T7
Glutathione peroxidase	At2g31570	P1B12	190H7T7
Glutathione reductase	At3g24170	P1C01	185P3T7
Photosystem II type I chlorophyll a /b binding protein;	At2g34430	P1C02	161I22T7
Putative [Mn] superoxide dismutase;	At3g10920	P1C03	109J19T7
Allene oxide synthase	At5g42650	P1C04	230J8T7
Catalase 1	At1g20630	P1C05	118M15T7
Glutaredoxin-like protein	At5g40370	P1C06	34D1T7
Adenosine-5'-phosphosulfate reductase	At1g62180	P1C07	73F9T7
Symbiosis-related like protein	At4g16520	P1C08	164P11T7
Wall-associated kinase 2	At1g21270	P1C09	230O3T7
Glutathione peroxidase; ATGP1	At4g11600	P1C10	118F4T7
GF14; F3F9.16; GF14omega isoform	At1g78300	P1C11	173H17T7
AHP1; ATHP3; histidine-containing phosphotransfer protein	At3g21510	P1C12	245J14T7
AHP2; ATHP1; histidine-containing phosphotransfer protein	At3g29350	P1D01	213J6T7
AHP3; ATHP2; histidine-containing phosphotransfer protein	At5g39340	P1D02	110E19T7
ETR1; ethylene-response protein	At1g66340	P1D03	89B11T7
Putative ethylene receptor (EIN4)	At3g04580	P1D04	245L12T7
Putative histidine kinase	At2g01830	P1D05	89M16T7

ARR1 protein;	At3g16857	P1D06	176M14T7
Response regulator 4	At1g10470	P1D07	242D6T7
Response regulator 5	At3g48100	P1D08	105E4T7
Response regulator 6	At5g62920	P1D09	138J22T7
Germin-like protein 1	At1g72610	P1D10	249C3T7
Germin-like protein 3b	At5g20630	P1D11	111F21T7
Germin-like protein 4	At1g18970	P1D12	114H1T7
Germin-like protein	At1g09560	P1E01	272H2T7
Germin-like protein 6	At5g39100	P1E02	157N14T7
Germin-like protein 7	At1g10460	P1E03	157B12T7
Germin-like protein 8	At3g05930	P1E04	125O15T7
Germin-like protein 9	At3g14630	P1E05	223N10T7
Germin-like protein 10	At1g08500	P1E06	269F10T7
Putative ethylene receptor; ETR2	At3g23150	P1E07	91N5T7
Cinnamyl-alcohol dehydrogenase CAD1	At4g39330	P1E08	119G15T7
Methyltransferase	At1g67980	P1E09	132K23T7
O-methyltransferase 1	At5g54160	P1E10	160E22T7
Chorismate mutase 3	At1g69370	P1E11	145F17T7
CYP83B1	At4g31500	P1E12	160M24T7
Phenyalanine ammonia-lyase	At3g53260	P1F01	CD3-122
Phenyalanine ammonia-lyase	At5g04230	P1F02	CD3-123
PAL1; phenylalanine ammonia lyase	At2g37040	P1F03	CD3-19
4-Coumarate--coenzyme A ligase (4CL)	At1g51680	P1F04	G2D3T7
Lipoxygenase 1	At1g55020	P1F05	H3C1T7
Lipoxygenase 2	At3g45140	P1F06	L23968
MAP kinase kinase kinase	At4g08500	P1F07	23A12T7
MAP kinase; mitogen-activated protein kinase 3	At3g45640	P1F08	77D1T7
Hypothetical protein T8M16_190	At3g56860	P1F09	78G4T7
Phosphoinositide-specific phospholipase C	At4g38530	P1F10	135N17T7
1-Phosphatidylinositol-4; 5-bisphosphate phosphodiesterase-like protein	At3g47290	P1F11	284C11T7
Chlorophyll a/b-binding protein type I	At2g34420	P1F12	170L24T7
Ethylene responsive element binding factor	At5g61590	P1G01	215C17T7
Receiver-like protein 3	At1g67710	P1G02	G9E6T7
ARR2 protein	At4g16110	P1G03	H3A8T7
Response reactor 3		P1G04	ATTS1197
Transcription factor inhibitor I kappa B	At1g64280	P1G05	99O5T7
Non-race specific disease resistance protein	At3g20600	P1G06	95G3T7
F2401.11; ACC oxidase	At1g62380	P1G07	G2B6T7

ACC-synthase-1	At3g61510	P1G08	U26543
ACC-synthase-2	At1g01480	P1G09	M95595
ACC-synthase-4	At2g22810	P1G10	U23481
ACC-synthase-5	At5g65800	P1G11	L29261
ACC-synthase-6	At4g11280	P1G12	U73786
Histidine kinase	At1g27320	P1H01	H10B9T7
AtERF4 (AP2-RAP2.5)	At3g15210	P1H02	227N9T7
Ethylene response sensor; ERS	At2g40940	P1H03	G5H10T7
Ethylene response sensor 2	At1g04310	P1H04	G12A3T7
Putative ABC transporter	At1g59870	P1H05	(GSF2_c01)
Putative vacuolar sorting receptor	At1g30900	P1H06	(GSF3_c06)
Stratagene Alien CDNA2(Control gene)	At3g60820	P1H07	
Myrosinase binding protein(JIP)	At3g16450	P1H08	(GSF1_a10)
Hypothetical protein	At2g32340	P1H09	(GSF1_c05)
pEARLI1	At4g12480	P1H10	(GSF1_f01) ?
H+-ATPase; plasma membrane H+-ATPase gene	At4g30190	P1H11	(GSF1_g03)?
PH_C_20 putative protein	At3g53990	P1H12	(GSF1_g05
Receptor-like protein kinase	At5g48380	P2A01	(GSF2_f02)
Succinate dehydrogenase iron-protein subunit	At3g27380	P2A02	(GSF2_f01)
Adenine phosphoribosyltransferase	At1g27450	P2A03	(GSF3_A06)
Copper/zinc superoxide dismutase	At2g28190	P2A04	(GSF1_h01)?
T16B5 unknown protein	At1g10760	P2A05	(GSF1_h10)
Low temperature and salt responsive protein homolog	At4g30650	P2A06	(GSF2_d01)
F13O11 hypothetical protein	At1g64720	P2A07	(GSF3_d07)
Salt-stress induced tonoplast intrinsic protein	At3g26520	P2A08	(GSF3_c04)
Metallothionein	At1g07610	P2A09	L15389
Cytochrome P450	At2g24180	P2A10	226P8T7
Lipase; R gene	At3g48090	P2A11	133C9T7
Myrosinase binding protein-like(JIP)	At3g16470	P2A12	95C9T7
Alternative oxidase	At3g22370	P2B01	127M17T7
Berberine bridge enzyme-like protein	At4g20860	P2B02	F14356
Catalase	At1g20620	P2B03	(GSF1_g07)?
Putative copper/zinc superoxide dismutase	At1g08830	P2B04	CD3-2
Fe-superoxide dismutase	At4g25100	P2B05	E6E5T7
Glutaredoxin (5'-adenylylsulfate reductase)	At4g04610	P2B06	ATTS3805
Glutathione peroxidase 1	At2g25080	P2B07	121P8T7
Glutathione S-transferase	At1g02920	P2B08	134B20T7
MRP-like ABC transporter	At2g34660	P2B09	164N3T7

Glutathione- S- transferase	At1g02930	P2B10	
Stress-induced protein OZI1 precursor	At4g00860	P2B11	20C9T7
PR1	At2g14610	P2B12	
Beta-1; 3-glucanase 2 (PR2)	At3g57260	P2C01	NP031928
Thaumatin-like protein(PR5)	At1g75040	P2C02	NP031927
Beta-1; 3-glucanase 2 (BG2)	At3g57260	P2C03	NP031928
Beta-1;3-glucanase(BG3)	At3g57240	P2C04	M58464
Acidic endochitinase	At5g24090	P2C05	145B23T7
Chitinase	At3g54420	P2C06	64H5T7
Tyrosine decarboxylase	At2g20340	P2C07	
Anionic peroxidase	At4g36430	P2C08	AT4g36430
S-adenosyl-L-homocysteine hydrolase (SHH)		P2C09	
Polygalacturonase inhibiting protein 2; PGIP2	At5g06870	P2C10	130I1T7
Arabinogalactan protein^^putative proline-rich protein	At2g14890	P2C11	167J15T7
Pectate lyase	At3g07010	P2C12	226D20T7
Pectin methyl esterase	At1g53840	P2D01	G6C7T7
Putative acetone-cyanohydrin lyase	At2g23600	P2D02	210E1T7
LRR-containing F-box protein	At2g39940	P2D03	92C23T7
AIG2 protein-like protein(AT3g28940)	At3g28940	P2D04	82H1T7
Hin1 homolog	At2g35980	P2D05	157G23T7
Thionin	At1g72260	P2D06	
Xyloglucan endo-1; 4-beta-D-glucanase-like protein;	At4g30280	P2D07	176K19T7
Similar to Rice cysteine proteinase inhibitor	At5g47550	P2D08	ATTS0249
Putative cysteine proteinase inhibitor B (cystatin B)	At2g40880	P2D09	ATTS0192
Protease inhibitor II	At2g02100	P2D10	178F24T7
Myrosinase binding protein-like(JIP)	At3g16420	P2D11	206L22T7
Putative transcription factor(MYB1)	At5g65790	P2D12	110F12T7
Remorin; DNA-binding protein	At2g45820	P2E01	144O8T7
14-3-3 Protein homologue (14-3-3a)	At5g16050	P2E02	220G10T7
Nitrilase; NIT3	At3g44320	P2E03	198M23T7
Cytochrome P450	At3g20130	P2E04	160C9T7
Glucose 6-phosphate dehydrogenase; clone E5	At5g13110	P2E05	42H10T7
Rotamase FKBP (ROF1)	At3g25230	P2E06	149C21T7
Methyltransferase	At1g67980	P2E07	132K23XP
Peroxidase like protein	At2g38380	P2E08	228O17T7
Putative polygalacuronase isoenzyme 1 beta subunit	At1g23760	P2E09	117N15T7
Delta-12 desaturase; Fad2	At3g12120	P2E10	135I5T7
Chalcone isomerase	At3g55120	P2E11	177A20T7

Pavonol synthase(AT5g08640)	At5g08640	P2E12	153O10T7
Putative NADPH oxidoreductase	At1g75280	P2F01	122B24XP
Flavanone 3-hydroxylase (FH3)	At3g51240	P2F02	123O20T7
Pre-hevein-like protein(HEL)	At3g04720	P2F03	200F6T7
Glutathione s-transferase	At1g02930	P2F04	206N21T7
Glutathione s-transferase	At2g29420	P2F05	103G1T7
Ascorbate peroxidase (thylakoid-bound)	At1g77490	P2F06	306C9T7
Vegetative storage protein VSP2	At5g24770	P2F07	67H8AT7
Cinamyl-alcohol dehydrogenase	At4g37980	P2F08	315D11T7
Anthranilate synthase alpha subunit	At5g05730	P2F09	240I16T7
Anthranilate synthase beta subunit 9	At1g25165	P2F10	241P6T7
Tryptophan synthase alpha subunit	At3g54640	P2F11	251L6T7
Tryptophan synthase beta subunit	At5g54810	P2F12	126P17T7
Steroid sulfotransferase-like protein	At5g07010	P2G01	23F3T7
Omega-3-desaturase	At3g11170	P2G02	36C4T7
Allene oxide synthase	At5g42650	P2G03	113C3XP
Putative wall-associated kinase 1	At1g21250	P2G04	39A3T7
Non-race specific disease resistance protein	At3g20600	P2G05	95G3T7
Calmodulin-2	At4g01150	P2G06	169J23T7
Rotamase fkbp homologue; calmodulin binding domain	At3g25230	P2G07	149C21T7
S-adenosylmethionine synthetase 2	At4g01850	P2G08	118N16T7
S-adenosyl-l-homocysteine hydrolase	At3g23810	P2G09	170K7T7
IAA-amino acid hydrolase homolog	At5g56650	P2G10	173C24T7
Vegetative storage protein (VSP1)	At5g24780	P2G11	114D3XP
Blue light photoreceptor/photolyase homolog	At1g04400	P2G12	G1D7T7
PWS1	Control	P2H01	
PWS2	Control	P2H02	
PWS3	Control	P2H03	
Single stranded DNA endonuclease RAD1	At5g41150	P2H04	
Type II CPD photolyase PHR1	At1g12370	P2H05	
Flavanoid-3'-hydroxylase	At4g10490	P2H06	
6-4 photolyase	At3g15620	P2H07	
Indole-3-acetate beta-glucosyltransferase		P2H08	
NADPH oxidase	At1g09100	P2H09	
Indole-3-acetate beta-glucosyltransferase like protein	At4g15480	P2H10	
Indole-3-acetate beta-glucosyltransferase like protein	At4g15490	P2H11	
Indole-3-acetate beta-glucosyltransferase	At4g15500	P2H12	

Table S2. DNA microarray analysis of transcripts in *Arabidopsis* suspension cell after NO treatment.

Gene#	Position	2h	SD	4h	SD	8h	SD	24h	SD
At3g13750	P0A01	2.97	0.26	2.20	0.17	1.88	0.22	1.99	0.49
At2g47580	P0A02	2.22	0.18	1.49	0.07	1.57	0.12	2.00	0.35
At2g39730	P0A03	2.52	0.22	1.36	0.09	1.20	0.53	1.75	0.59
At5g38430	P0A04	2.33	0.92	1.17	0.10	1.51	0.14	1.89	0.50
At3g29030	P0A05	2.76	0.51	1.35	0.09	1.53	0.11	2.07	0.63
At2g29330	P0A06	2.58	0.29	1.16	0.19	1.58	0.13	2.09	0.61
At5g38470	P0A07	2.39	0.19	1.71	0.15	1.89	0.12	1.96	0.29
At1g76890	P0A08	2.46	0.26	1.12	0.08	1.48	0.06	2.00	0.59
At5g55920	P0A09	2.39	0.43	1.20	0.09	1.47	0.04	2.00	0.53
At5g61820	P0A10	4.36	0.58	5.09	0.45	5.17	0.17	3.07	0.97
At1g64230	P0A11	2.23	0.09	1.78	0.09	1.96	0.12	1.65	0.25
At5g38410	P0A12	2.62	0.12	1.47	0.20	1.62	0.10	2.22	0.53
At5g08670	P0B01	1.73	0.06	1.13	0.04	1.38	0.07	2.05	0.40
At2g14100	P0B02	2.86	0.22	2.01	0.07	1.75	0.15	1.86	0.50
At5g49660	P0B03	1.93	0.08	1.74	0.02	1.70	0.10	1.93	0.12
At4g23220	P0B04	3.44	1.14	2.52	0.08	2.03	0.13	2.12	0.65
At1g77990	P0B05	2.65	1.03	1.36	0.08	1.32	0.11	1.73	0.62
At1g23130	P0B06	2.20	0.36	1.47	0.10	1.48	0.14	2.17	0.41
At2g21660	P0B07	1.82	0.09	1.23	0.07	1.50	0.04	1.75	0.27
At2g04780	P0B08	1.84	0.36	1.34	0.08	1.55	0.09	1.31	0.24
At2g21170	P0B09	2.01	0.30	1.24	0.07	1.28	0.09	1.57	0.36
At1g73740	P0B10	2.10	0.06	1.46	0.15	1.64	0.10	2.18	0.35
At4g18360	P0B11	2.21	0.35	1.36	0.08	1.50	0.07	2.05	0.41
At1g54270	P0B12	1.95	0.11	1.80	0.12	1.84	0.11	2.00	0.09
At2g36460	P0C01	4.22	1.18	2.48	0.06	1.98	0.17	2.04	1.05
At1g24180	P0C02	2.16	0.14	1.50	0.08	1.57	0.13	1.76	0.29
At1g73880	P0C03	3.15	0.31	1.46	0.84	1.66	0.10	1.98	0.76
At5g65050	P0C04	2.86	0.28	1.90	0.10	1.82	0.11	2.00	0.48
At3g08770	P0C05	2.89	0.81	1.46	0.11	1.55	0.11	1.72	0.67
At5g16470	P0C06	2.71	0.12	1.77	0.13	1.72	0.09	2.00	0.46
At1g06680	P0C07	2.67	0.52	1.49	0.14	1.63	0.05	1.71	0.54
At3g55120	P0C08	2.98	0.49	1.39	0.16	1.52	0.18	1.98	0.72
At1g65980	P0C09	2.56	0.16	2.25	0.15	1.94	0.10	1.39	0.50
At1g09210	P0C10	1.52	0.09	0.75	0.07	1.07	0.07	2.19	0.62
At5g09660	P0C11	2.85	0.66	1.52	0.20	1.61	0.10	2.20	0.61
At3g15000	P0C12	2.39	0.30	1.53	0.12	1.58	0.18	1.93	0.39
At1g79550	P0D01	2.43	0.07	1.80	0.04	2.04	0.14	1.85	0.29
At5g45710	P0D02	2.39	0.17	1.47	0.05	1.66	0.06	1.70	0.40
At2g45180	P0D03	1.99	0.07	1.58	0.11	1.47	0.08	1.77	0.23
At5g46110	P0D04	2.57	0.10	1.70	0.06	1.59	0.09	1.78	0.45
At5g40580	P0D05	2.10	0.04	1.48	0.07	2.10	0.15	1.76	0.30
At1g73330	P0D06	2.24	0.26	1.41	0.08	1.56	0.09	2.09	0.40
At5g10960	P0D07	1.76	0.11	1.38	0.06	1.43	0.08	1.89	0.25

At2g21660	P0D08	2.36	0.24	1.62	0.12	1.63	0.05	1.90	0.35
At5g54640	P0D09	2.08	0.20	1.31	0.05	1.46	0.08	1.81	0.35
At3g48990	P0D10	1.96	0.14	1.46	0.14	1.80	0.12	1.77	0.21
At5g48760	P0D11	1.91	0.06	1.11	0.08	1.18	0.06	2.30	0.58
At4g14130	P0D12	2.42	0.44	1.38	0.07	1.72	0.07	1.61	0.45
At3g09440	P0E01	4.27	0.43	3.68	0.19	2.95	0.15	1.08	1.39
At4g31720	P0E02	2.49	0.11	1.76	0.18	1.75	0.11	1.85	0.36
At1g75330	P0E03	2.16	0.14	1.44	0.11	1.49	0.13	1.98	0.36
At5g20700	P0E04	1.43	0.15	1.35	0.07	1.20	0.10	2.04	0.37
At1g67090	P0E05	2.75	0.88	1.31	0.15	1.52	0.08	1.78	0.64
At1g22300	P0E06	1.63	0.10	1.30	0.09	1.53	0.12	1.94	0.26
At1g05850	P0E07	1.86	0.12	1.37	0.09	1.27	0.09	1.35	0.27
At2g18110	P0E08	2.25	0.06	1.42	0.13	1.49	0.11	1.80	0.37
At5g20250	P0E09	2.83	0.36	1.70	0.14	1.72	0.18	1.87	0.54
At1g04100	P0E10	2.50	0.20	1.15	0.09	1.27	0.14	1.66	0.61
At5g03240	P0E11	2.21	0.31	2.57	0.13	2.23	0.11	1.89	0.28
At3g15210	P0E12	2.58	0.29	1.61	0.20	1.59	0.11	1.92	0.46
At3g56240	P1A01	1.26	0.15	0.96	0.06	1.26	0.18	1.21	0.15
At5g52310	P1A02	1.93	0.09	1.23	0.10	1.39	0.13	1.85	0.34
At5g44420	P1A03	1.97	0.48	1.00	0.21	1.34	0.15	1.06	0.45
At4g23300	P1A04	1.47	0.09	1.01	0.09	1.08	0.11	1.43	0.24
At1g30510	P1A05	1.28	0.09	1.01	0.09	0.99	0.20	1.48	0.24
At2g34430	P1A06	2.07	0.25	1.02	0.07	1.37	0.07	1.56	0.44
At1g05010	P1A07	1.87	0.14	1.17	0.07	1.32	0.11	1.50	0.30
	P1A08	1.96	0.27	0.80	0.15	1.13	0.10	1.53	0.50
At4g22330	P1A09	2.27	0.55	1.00	0.10	2.04	1.32	1.32	0.60
At2g40940	P1A10	1.95	0.77	0.75	0.14	1.25	0.10	1.24	0.49
At1g12270	P1A11	2.22	0.25	1.84	0.24	2.27	0.22	1.22	0.49
At1g31580	P1A12	2.42	0.89	1.12	0.11	1.23	0.46	1.34	0.60
At4g02520	P1B01	1.77	0.48	0.69	0.28	0.80	0.72	0.34	0.61
At5g13930	P1B02	8.97	11.75	0.88	0.08	1.26	0.09	1.24	3.93
At2g34420	P1B03	3.06	0.33	0.86	0.03	1.52	0.16	1.71	0.92
At1g77760	P1B04	2.32	0.78	1.75	0.14	1.80	0.06	2.01	0.26
At3g55430	P1B05	3.02	0.58	2.45	0.13	1.87	0.20	1.73	0.59
At1g07890	P1B06	3.26	0.46	2.26	0.31	1.98	0.17	1.42	0.77
At3g22370	P1B07	8.36	0.10	2.83	0.22	2.51	0.42	1.71	3.04
At3g01420	P1B08	2.43	0.27	3.46	0.41	2.22	0.25	4.57	1.08
At5g20230	P1B09	2.25	0.15	2.52	0.12	1.29	0.35	0.60	0.88
At3g49120	P1B10	3.81	0.65	2.38	0.27	1.92	0.20	0.81	1.24
At3g12500	P1B11	1.87	0.08	1.46	0.10	1.14	0.10	2.10	0.43
At2g31570	P1B12	2.13	0.18	2.73	0.28	2.84	0.27	2.22	0.36
At3g24170	P1C01	2.06	0.17	1.55	0.04	1.92	0.21	1.64	0.24
At2g34430	P1C02	2.00	0.21	1.30	0.08	1.11	0.70	1.62	0.39
At3g10920	P1C03	2.12	0.31	0.98	0.11	1.29	0.07	1.50	0.48
At5g42650	P1C04	3.30	0.12	1.48	0.18	1.77	0.33	1.47	0.87
At1g20630	P1C05	2.25	0.48	2.21	0.09	2.74	0.20	2.04	0.30
At5g40370	P1C06	2.17	0.17	1.98	0.19	2.83	0.11	2.16	0.37
At1g62180	P1C07	4.95	0.55	2.00	0.12	1.59	0.15	1.37	1.67
At4g16520	P1C08	2.42	0.17	1.95	0.10	2.10	0.10	2.01	0.21

At1g21270	P1C09	4.24	1.01	0.99	0.11	1.23	0.38	1.21	1.55
At4g11600	P1C10	3.58	0.21	4.23	0.30	4.28	0.16	1.71	1.20
At1g78300	P1C11	2.12	0.20	1.46	0.14	1.73	0.12	1.77	0.27
At3g21510	P1C12	2.29	0.21	1.65	0.39	1.94	0.09	2.17	0.28
At3g29350	P1D01	1.84	0.16	1.19	0.15	1.60	0.09	1.61	0.27
At5g39340	P1D02	1.85	0.12	1.38	0.09	1.84	0.12	1.68	0.22
At1g66340	P1D03	1.99	0.47	1.04	0.06	1.32	0.13	1.48	0.40
At3g04580	P1D04	2.03	0.13	1.00	0.10	1.36	0.08	1.49	0.43
At2g01830	P1D05	4.01	2.38	0.87	0.06	1.42	0.12	1.20	1.44
At3g16857	P1D06	2.26	0.44	1.14	0.07	1.49	0.14	1.76	0.47
At1g10470	P1D07	2.29	0.28	1.33	0.07	1.39	0.14	1.74	0.44
At3g48100	P1D08	3.44	0.58	1.00	0.13	1.25	0.22	1.09	1.17
At5g62920	P1D09	3.62	0.61	1.14	0.06	1.33	0.06	1.34	1.18
At1g72610	P1D10	5.82	4.77	0.75	0.21	0.96	0.09	0.95	2.47
At5g20630	P1D11	1.28	14.61	0.70	0.16	0.94	0.27	1.02	0.24
At1g18970	P1D12	3.51	1.64	0.98	0.23	1.25	0.18	3.29	1.33
At1g09560	P1E01	2.00	0.11	1.50	0.07	1.35	0.28	2.36	0.46
At5g39100	P1E02	-2.42	11.47	0.99	0.08	1.24	0.10	1.04	1.76
At1g10460	P1E03	5.80	3.27	0.88	0.08	1.05	0.10	1.34	2.36
At3g05930	P1E04	2.72	0.14	1.75	0.22	1.48	0.07	1.21	0.66
At3g14630	P1E05	2.77	0.34	1.68	0.08	1.44	0.06	0.92	0.78
At1g08500	P1E06	3.27	1.80	0.72	0.14	1.16	0.24	0.83	1.20
At3g23150	P1E07	2.02	0.28	1.05	0.04	1.10	0.09	1.53	0.45
At4g39330	P1E08	2.44	0.24	1.57	0.16	1.82	0.10	1.82	0.37
At1g67980	P1E09	5.72	3.76	1.07	0.53	1.35	0.12	1.36	2.24
At5g54160	P1E10	2.28	0.45	1.21	0.14	1.39	0.21	0.85	0.61
At1g69370	P1E11	5.25	3.94	1.03	0.11	1.27	0.05	1.40	2.01
At4g31500	P1E12	0.73	0.73	0.49	0.08	0.47	0.03	0.66	0.13
At3g53260	P1F01	2.36	0.70	0.91	0.19	1.60	0.73	1.07	0.65
At5g04230	P1F02	2.28	0.36	0.86	0.08	1.29	0.13	1.56	0.60
At2g37040	P1F03	2.34	0.28	1.29	0.12	1.52	0.17	1.85	0.45
At1g51680	P1F04	2.32	0.32	1.37	0.14	1.63	0.08	1.98	0.41
At1g55020	P1F05	2.48	0.46	1.78	0.10	1.69	0.12	1.62	0.40
At3g45140	P1F06	2.55	0.56	1.32	0.11	1.57	0.12	1.51	0.55
At4g08500	P1F07	#####	#####	0.80	0.16	1.34	0.06	1.19	#####
At3g45640	P1F08	2.89	0.34	1.22	0.18	1.31	0.01	1.34	0.80
At3g56860	P1F09	2.82	0.50	1.13	0.09	1.39	0.07	1.79	0.75
At4g38530	P1F10	3.09	0.58	1.04	0.18	1.29	0.17	1.02	1.00
At3g47290	P1F11	1.97	0.29	1.07	0.03	1.20	0.09	1.59	0.40
At2g34420	P1F12	2.35	0.34	1.45	0.15	1.65	0.05	0.47	0.78
At5g61590	P1G01	3.77	1.79	1.29	0.10	1.18	0.78	1.73	1.21
At1g67710	P1G02	3.45	0.92	1.04	0.10	1.30	0.09	1.58	1.09
At4g16110	P1G03	3.33	0.61	0.99	0.05	1.31	0.09	1.41	1.06
	P1G04	2.36	0.29	1.05	0.12	1.36	0.04	1.75	0.57
At1g64280	P1G05	2.89	0.51	1.10	0.08	1.24	0.12	1.50	0.82
At3g20600	P1G06	2.40	0.45	1.02	0.09	1.35	0.14	1.68	0.59
At1g62380	P1G07	1.90	0.18	0.69	0.02	1.13	0.05	2.34	0.74
At3g61510	P1G08	5.30	1.08	1.01	0.20	1.39	0.06	1.13	2.07
At1g01480	P1G09	#####	#####	0.74	0.73	1.05	0.15	0.59	#####

At2g22810	P1G10	1.72	2.67	0.75	0.17	0.94	0.20	1.18	0.42
At5g65800	P1G11	-1.81	1.01	0.43	0.23	0.86	0.11	0.76	1.26
At4g11280	P1G12	0.22	6.05	4.02	6.44	1.03	0.21	0.62	1.73
At1g27320	P1H01	1.43	0.79	1.17	0.18	1.51	0.18	0.94	0.26
At3g15210	P1H02	2.14	0.40	1.16	0.13	1.11	0.20	1.56	0.48
At2g40940	P1H03	#####	#####	0.73	0.12	1.16	0.14	1.80	#####
At1g04310	P1H04	5.68	3.17	1.03	0.08	1.12	0.05	1.76	2.21
At1g59870	P1H05	3.58	0.45	0.90	0.35	0.93	0.12	1.14	1.30
At1g30900	P1H06	2.96	0.72	0.77	0.17	0.96	0.20	1.33	1.00
At3g60820	P1H07	2.03	0.20	1.34	0.09	1.94	0.13	1.68	0.31
At3g16450	P1H08	1.91	0.31	1.22	0.14	1.21	0.09	1.88	0.39
At2g32340	P1H09	3.28	2.01	0.95	0.04	1.60	0.09	1.45	1.02
At4g12480	P1H10	3.03	0.25	1.14	0.23	1.30	0.21	3.94	1.36
At4g30190	P1H11	3.91	2.80	0.86	0.13	1.05	0.06	1.92	1.39
At3g53990	P1H12	2.32	0.40	3.51	0.43	3.10	0.23	1.37	0.95
At5g48380	P2A01	2.29	0.25	0.66	0.11	0.99	0.08	1.00	0.72
At3g27380	P2A02	1.94	0.22	1.06	0.10	0.96	0.59	1.74	0.49
At1g27450	P2A03	1.85	0.08	0.94	0.05	1.18	0.07	1.55	0.40
At2g28190	P2A04	2.44	0.32	0.77	0.13	1.11	0.09	1.61	0.73
At1g10760	P2A05	1.95	0.27	0.64	0.11	1.02	0.08	1.11	0.55
At4g30650	P2A06	2.40	0.19	0.52	0.14	0.85	0.05	1.03	0.82
At1g64720	P2A07	2.32	0.35	0.62	0.06	1.05	0.08	0.95	0.75
At3g26520	P2A08	1.90	0.26	0.77	0.17	0.86	0.08	0.90	0.53
At1g07610	P2A09	1.86	0.34	3.33	0.11	4.19	0.31	3.61	0.99
At2g24180	P2A10	2.61	0.26	1.25	0.15	1.46	0.11	1.80	0.60
At3g48090	P2A11	2.03	0.08	1.43	0.18	1.68	0.07	1.23	0.35
At3g16470	P2A12	2.96	0.48	1.08	0.12	1.20	0.03	1.84	0.86
At3g22370	P2B01	6.71	0.16	2.01	0.16	1.90	0.37	1.50	2.46
At4g20860	P2B02	2.77	0.15	0.78	0.08	1.08	0.11	1.42	0.88
At1g20620	P2B03	2.18	0.19	0.78	0.03	1.22	0.12	1.16	0.60
At1g08830	P2B04	1.78	0.16	0.99	0.14	1.28	0.08	1.27	0.33
At4g25100	P2B05	2.49	0.31	0.56	0.03	0.83	0.22	1.04	0.86
At4g04610	P2B06	3.01	0.09	1.45	0.11	1.29	0.10	1.39	0.82
At2g25080	P2B07	2.33	0.23	2.24	0.04	2.55	0.11	1.34	0.53
At1g02920	P2B08	3.58	0.19	4.47	0.29	1.93	0.19	0.60	1.72
At2g34660	P2B09	4.17	0.13	2.57	0.05	2.79	0.12	1.79	0.99
At1g02930	P2B10	3.08	0.22	1.99	0.17	1.58	0.07	1.26	0.79
At4g00860	P2B11	1.48	0.02	1.35	0.10	1.71	0.14	2.19	0.37
At2g14610	P2B12	2.90	0.31	1.04	0.20	1.38	0.15	1.69	0.81
At3g57260	P2C01	1.91	0.19	0.87	0.16	1.19	0.09	0.92	0.48
At1g75040	P2C02	2.56	0.50	0.90	0.09	1.14	0.04	2.02	0.77
At3g57260	P2C03	2.85	0.34	0.48	0.06	0.97	0.18	0.82	1.07
At3g57240	P2C04	2.24	0.07	1.16	0.07	1.54	0.08	2.85	0.75
At5g24090	P2C05	2.15	0.20	1.04	0.03	1.51	0.04	2.20	0.55
At3g54420	P2C06	2.13	0.31	0.80	0.14	1.08	0.09	1.38	0.57
At2g20340	P2C07	2.02	0.17	1.08	0.05	1.46	0.09	1.35	0.39
At4g36430	P2C08	2.17	0.30	0.78	0.28	1.04	0.25	1.62	0.62
	P2C09	1.67	0.07	0.63	0.10	0.77	0.05	0.93	0.46
At5g06870	P2C10	1.90	0.02	1.08	0.08	0.83	0.09	2.29	0.68

At2g14890	P2C11	1.86	0.15	1.28	0.06	1.72	0.11	1.55	0.25
At3g07010	P2C12	2.02	0.48	1.19	0.19	1.34	0.11	1.64	0.37
At1g53840	P2D01	1.57	0.15	0.70	0.16	1.10	0.05	0.89	0.38
At2g23600	P2D02	2.60	0.23	0.93	0.06	1.30	0.11	1.49	0.72
At2g39940	P2D03	1.54	0.02	0.90	0.05	0.92	0.08	1.56	0.37
At3g28940	P2D04	1.71	0.15	1.17	0.07	1.03	0.06	1.85	0.40
At2g35980	P2D05	3.03	0.40	2.02	0.19	1.54	0.05	1.44	0.73
At1g72260	P2D06	2.17	0.41	0.86	0.14	1.23	0.08	2.05	0.63
At4g30280	P2D07	1.32	0.19	1.35	0.06	1.62	0.07	1.34	0.14
At5g47550	P2D08	2.02	0.28	1.17	0.13	1.31	0.10	1.79	0.40
At2g40880	P2D09	2.69	0.41	0.86	0.01	1.34	0.08	1.28	0.79
At2g02100	P2D10	1.98	0.08	1.05	0.09	1.31	0.10	1.57	0.40
At3g16420	P2D11	1.52	0.12	1.25	0.06	0.96	0.08	2.04	0.46
At5g65790	P2D12	2.21	0.06	0.93	0.15	1.62	0.14	2.12	0.59
At2g45820	P2E01	1.76	0.13	1.31	0.06	1.58	0.08	1.51	0.18
At5g16050	P2E02	2.16	0.11	1.77	0.09	1.85	0.11	1.84	0.17
At3g44320	P2E03	2.34	0.07	1.94	0.05	1.77	0.08	1.89	0.25
At3g20130	P2E04	2.19	0.30	1.42	0.09	1.41	0.12	1.88	0.38
At5g13110	P2E05	2.35	0.05	1.58	0.04	1.34	0.06	1.46	0.46
At3g25230	P2E06	1.78	0.07	1.49	0.08	1.51	0.10	1.58	0.13
At1g67980	P2E07	3.35	0.35	1.40	0.25	1.27	0.06	1.43	1.00
At2g38380	P2E08	2.72	0.13	2.14	0.04	3.75	0.44	10.05	3.65
At1g23760	P2E09	1.96	0.07	1.00	0.08	1.32	0.12	1.76	0.43
At3g12120	P2E10	1.98	0.10	1.45	0.10	1.25	0.13	1.15	0.37
At3g55120	P2E11	2.07	0.18	1.17	0.02	1.35	0.07	2.57	0.65
At5g08640	P2E12	2.15	0.16	1.27	0.18	1.41	0.19	1.83	0.40
At1g75280	P2F01	3.71	0.18	2.55	0.34	1.88	0.09	1.52	0.96
At3g51240	P2F02	2.12	0.23	1.77	0.12	1.07	0.04	3.03	0.81
At3g04720	P2F03	3.13	0.44	3.48	0.16	1.44	0.12	1.16	1.17
At1g02930	P2F04	4.01	0.21	4.46	0.23	3.20	0.18	1.07	1.50
At2g29420	P2F05	5.92	0.18	4.54	0.33	4.23	0.13	2.28	1.50
At1g77490	P2F06	2.33	0.46	1.22	0.08	1.37	0.11	1.79	0.50
At5g24770	P2F07	2.39	0.25	0.95	0.10	1.23	0.06	1.52	0.62
At4g37980	P2F08	2.14	0.43	1.57	0.23	1.60	0.21	1.94	0.27
At5g05730	P2F09	2.13	0.03	1.16	0.03	1.13	0.11	1.59	0.47
At1g25165	P2F10	1.26	0.23	0.93	0.12	0.92	0.12	1.25	0.19
At3g54640	P2F11	1.30	0.20	0.84	0.08	0.76	0.10	1.08	0.24
At5g54810	P2F12	1.88	0.04	1.21	0.15	1.20	0.13	1.60	0.33
At5g07010	P2G01	1.34	0.20	0.94	0.05	1.14	0.05	2.27	0.59
At3g11170	P2G02	1.76	0.10	0.92	0.16	0.95	0.04	1.28	0.39
At5g42650	P2G03	2.55	0.38	0.91	0.11	1.20	0.07	1.53	0.71
At1g21250	P2G04	2.25	0.46	1.09	0.09	1.24	0.18	1.73	0.53
At3g20600	P2G05	2.49	0.28	1.06	0.04	1.33	0.07	1.76	0.62
At4g01150	P2G06	1.92	0.18	1.30	0.12	1.39	0.09	1.98	0.35
At3g25230	P2G07	2.48	0.10	2.08	0.12	1.90	0.11	1.71	0.33
At4g01850	P2G08	1.18	0.04	0.80	0.03	1.02	0.05	0.86	0.17
At3g23810	P2G09	1.70	0.08	0.85	0.05	0.88	0.08	0.96	0.40
At5g56650	P2G10	1.93	0.07	1.33	0.11	1.38	0.11	1.90	0.33
At5g24780	P2G11	2.92	1.06	0.99	0.05	1.26	0.08	1.98	0.86

At1g04400	P2G12	2.00	0.03	1.26	0.30	1.43	0.14	2.06	0.40
Control	P2H01	2.80	0.09	0.84	0.05	1.24	0.10	1.42	0.85
Control	P2H02	2.14	0.05	1.06	0.04	1.29	0.09	2.06	0.54
Control	P2H03	2.41	0.09	0.93	0.05	1.28	0.08	1.60	0.63
At5g41150	P2H04	2.41	0.50	1.12	0.13	1.39	0.16	2.53	0.71
At1g12370	P2H05	2.41	0.33	0.96	0.07	1.20	0.12	1.66	0.64
At4g10490	P2H06	2.78	0.31	1.06	0.28	1.26	0.19	2.52	0.87
At3g15620	P2H07	2.54	0.35	1.19	0.06	1.33	0.05	2.02	0.63
	P2H08	2.07	0.04	1.40	0.15	1.46	0.13	2.65	0.59
At1g09100	P2H09	2.36	0.20	1.04	0.13	1.28	0.11	1.76	0.58
At4g15480	P2H10	3.03	0.44	1.13	0.16	1.34	0.09	1.84	0.85
At4g15490	P2H11	2.17	0.44	1.11	0.07	1.30	0.11	2.00	0.52
At4g15500	P2H12	2.29	0.19	1.28	0.30	1.48	0.16	2.14	0.49

The genes with weak signals (lower than 2 fold local background) were neglected by grey scripts. The genes with induction over 3.5 fold are marked by red colored boxes, the ratio below 0.5 are marked with green boxes, and with induction between 2.5-3.5 fold by orange colored boxes. Array data were based on four independent replicates and the standard deviation (SD) were shown on the right side of each ratio.

Table S3. DNA microarray analysis of transcripts in *Arabidopsis* plants after gaseous NO treatment.

Gene	Position	1h Col.	SD	3h Col.	SD	1h NahG	SD	3h NahG	SD	1h jar1	SD	3h jar1	SD
At2g47580	D7A01	1.27	0.15	1.16	0.05	1.67	0.42	0.87	0.22	0.85	0.45	2.21	0.15
At2g39730	D7A02	1.07	0.43	0.96	0.15	2.20	0.49	2.16	1.44	1.55	0.86	0.53	0.06
At5g38430	D7A03	0.98	0.02	0.86	0.15	1.50	0.11	1.21	0.42	1.24	0.37	0.77	0.06
At5g41700	D7A04	3.32	0.52	2.85	0.13	1.22	0.16	1.32	0.58	1.54	0.27	1.04	0.11
At5g38410	D7A05	0.99	0.11	0.84	0.23	1.51	0.12	1.14	0.35	1.17	0.29	0.91	0.07
At5g08670	D7A06	1.57	0.45	1.39	0.14	1.64	0.20	1.46	0.49	1.33	0.64	0.58	0.07
At2g21170	D7A07	0.97	0.31	0.81	0.07	1.95	0.41	0.98	0.13	1.07	0.44	1.26	0.31
At4g18360	D7A08	1.09	0.30	1.00	0.05	1.79	0.34	0.96	0.42	0.77	0.32	1.04	0.09
At1g06680	D7A09	1.35	0.37	1.19	0.12	1.43	0.19	1.00	0.36	1.50	0.65	0.78	0.1
At5g38420	D7A10	1.43	0.48	1.25	0.20	1.17	0.19	0.90	0.29	1.29	0.29	0.71	0.1
At1g67090	D7A11	0.98	0.03	0.87	0.18	1.35	0.20	1.08	0.36	1.25	0.46	0.9	0.14
At5g03240	D7A12	1.46	0.18	1.30	0.13	1.45	0.37	1.73	0.65	1.41	0.24	0.92	0.2
At3g10230	D7B01	1.14	0.43	1.01	0.17	1.20	0.22	1.10	0.51	0.84	0.34	0.73	0.11
At5g57030	D7B02	0.50	0.28	0.48	0.05	1.67	0.38	1.55	0.90	0.79	0.88	0.52	0.06
At5g52570	D7B03	0.54	0.28	0.53	0.04	1.27	0.08	0.98	0.35	0.96	0.53	0.64	0.08
At5g67030	D7B04	0.64	0.25	0.53	0.16	1.73	0.46	1.55	0.93	0.84	0.74	0.54	0.04
At1g08550	D7B05	0.69	0.31	0.53	0.23	0.86	0.56	1.19	0.77	2.13	3.01	0.62	0.06
At5g43860	D7B07	0.73	0.53	0.61	0.40	1.68	0.35	1.43	0.91	0.96	0.94	0.66	0.06
At4g37000	D7B08	0.68	0.18	0.53	0.06	0.64	1.11	1.17	0.54	1.01	0.50	0.63	0.08
At4g36220	D7B09	0.68	0.13	0.57	0.07	1.26	0.14	1.38	0.82	0.54	0.25	0.58	0.06
At3g52430	D7B11	0.67	0.07	0.53	0.09	1.28	0.37	1.21	0.48	1.02	0.35	0.78	0.1
At5g15410	D7B12	0.41	0.11	0.34	0.10	2.95	2.20	2.78	1.14	0.82	0.71	0.68	0.14
At4g36380	D7C01	2.12	0.16	1.81	0.04	1.36	0.09	1.05	0.31	1.35	0.24	1.05	0.06
At4g09320	D7C02	2.47	0.38	2.04	0.04	1.37	0.22	1.33	0.15	1.14	0.45	0.83	0.09
At1g75910	D7C03	2.89	1.07	2.51	0.66	1.27	0.18	1.51	0.43	1.44	0.39	1.6	0.1
At1g78820	D7C04	1.62	0.49	1.38	0.15	2.26	0.49	1.38	0.34	1.76	0.52	1.42	0.08
At2g30870	D7C05	2.59	0.15	2.06	0.37	3.36	1.59	2.85	0.60	2.11	0.64	1.55	0.11
At2g44500	D7C06	1.91	0.62	1.56	0.22	1.02	0.17	1.46	0.44	1.35	0.42	1.73	0.14
At5g55190	D7C07	1.40	0.28	1.12	0.55	1.54	0.28	1.32	0.33	1.29	0.38	0.62	0.1
At3g47800	D7C08	1.15	0.01	0.97	0.15	4.09	1.55	5.35	1.56	1.69	1.21	14.8	3.46
At3g56800	D7C09	3.70	1.41	3.19	1.68	1.76	0.20	1.26	0.52	2.05	0.53	0.86	0.1
At3g56940	D7C10	0.98	0.11	0.82	0.19	2.84	0.91	2.41	0.83	1.77	0.91	0.99	0.09
At2g45960	D7C11	1.29	0.42	1.22	0.36	1.02	0.15	1.49	0.50	1.22	0.28	1.32	0.12
At1g76100	D7C12	1.31	0.48	1.12	0.13	1.37	0.13	0.96	0.25	1.32	0.53	0.9	0.06
At2g40000	D7D01	0.30	0.08	0.42	0.14	2.86	0.91	1.23	0.08	1.34	0.74	0.49	0.1
At1g65660	D7D02	0.72	0.11	0.60	0.05	1.39	0.10	1.14	0.30	1.46	0.34	1.01	0.09
At3g44310	D7D03	0.95	0.15	0.83	0.03	1.57	0.21	1.27	0.46	2.44	2.36	1.42	0.12
At2g41430	D7D04	5.73	4.74	5.04	4.53	7.48	2.51	1.41	0.45	5.79	5.82	1.39	0.08
At3g47833	D7D05	2.75	0.73	2.77	0.47	1.38	0.09	1.30	0.57	1.51	0.13	1.35	0.09
At1g27730	D7D06	1.31	0.78	1.09	0.67	2.57	0.98	1.64	0.54	2.50	1.28	1.3	0.09
At2g19810	D7D07	2.21	0.04	1.87	0.20	1.42	0.24	1.16	0.19	1.50	0.23	1.22	0.08
At4g30530	D7D08	3.05	1.91	2.50	1.76	6.36	3.85	2.26	0.49	3.92	3.27	1.19	0.09
At5g01410	D7D09	1.00	0.14	0.85	0.00	1.63	0.33	1.62	0.66	1.34	0.33	1.12	0.1
At5g09650	D7D10	1.36	0.06	1.11	0.14	1.36	0.22	1.26	0.37	1.20	0.28	0.92	0.11
At1g01620	D7D11	1.12	0.00	0.92	0.14	0.95	0.15	1.24	0.48	1.37	0.36	1.19	0.09

At1g78300	D7D12	2.48	0.15	2.19	0.34	1.44	0.28	1.24	0.42	1.41	0.31	0.85	0.07
At1g24180	D7E01	1.43	0.32	1.28	0.24	1.39	0.11	1.34	0.40	1.17	0.38	1.29	0.05
At5g63310	D7E02	0.95	0.11	0.82	0.19	1.46	0.16	1.19	0.40	1.14	0.33	0.67	0.04
At5g57940	D7E03	1.05	0.35	0.83	0.16	1.47	0.18	1.28	0.38	1.46	0.20	1.39	0.07
At3g17760	D7E04	0.55	0.10	0.53	0.07	1.38	0.21	1.45	0.66	1.28	0.46	1.27	0.09
At4g16190	D7E05	1.41	0.40	1.17	0.13	1.39	0.25	1.56	0.67	1.34	0.21	1.32	0.09
At3g01120	D7E06	1.07	0.19	0.90	0.09	1.28	0.12	1.53	0.38	1.31	0.29	1.27	0.08
At5g47520	D7E07	1.92	0.22	1.42	0.07	1.38	0.20	1.51	0.34	1.59	0.32	1.23	0.08
At1g08550	D7E08	0.79	0.05	0.68	0.09	1.31	0.14	1.34	0.62	1.56	0.85	1.21	0.09
At3g54110	D7E09	1.59	0.46	1.26	0.08	1.59	0.38	1.06	0.08	1.25	0.33	1.53	0.13
At3g16857	D7E10	1.33	0.32	1.13	0.18	1.63	0.30	1.36	0.44	1.23	0.89	0.64	0.08
At2g21250	D7E11	0.80	0.10	0.70	0.03	1.34	0.22	1.27	0.50	1.75	0.71	1.79	0.14
At5g05110	D7E12	1.31	0.32	1.17	0.14	1.41	0.08	1.40	0.48	0.97	0.59	0.91	0.09
At4g34710	D7F01	1.57	0.33	1.34	0.09	3.17	1.37	1.70	0.09	1.32	0.78	2.57	0.19
At5g15970	D7F02	7.60	1.27	5.95	0.22	2.55	1.60	2.16	0.56	3.52	1.74	2.96	0.28
At5g50370	D7F03	0.76	0.11	0.71	0.24	1.49	0.65	1.34	0.42	1.33	0.71	1.34	0.07
At3g50000	D7F04	1.27	0.21	1.07	0.10	1.10	0.94	1.39	0.54	1.50	0.21	1.26	0.13
At1g32370	D7F05	1.39	0.33	1.14	0.01	1.87	0.51	0.80	0.17	1.36	0.21	1.29	0.08
At5g05700	D7F06	1.59	0.51	1.37	0.27	1.68	0.12	1.31	0.22	1.32	0.14	1.3	0.08
At4g21960	D7F07	0.82	0.26	0.69	0.06	1.84	0.63	2.28	1.44	1.52	0.71	1.17	0.08
At1g05850	D7F08	0.79	0.08	0.66	0.03	1.47	0.30	1.25	0.49	1.56	0.18	1.1	0.07
At3g48000	D7F09	0.84	0.00	0.75	0.16	3.96	3.26	1.86	0.68	1.42	0.27	1.12	0.1
At5g05170	D7F10	1.77	0.22	1.50	0.01	2.34	0.35	1.46	0.57	2.12	1.32	0.59	0.04
At2g30860	D7F11	1.02	0.09	0.86	0.01	1.64	0.44	2.26	0.63	1.67	0.43	3.73	0.3
At1g13450	D7F12	1.28	0.52	1.09	0.26	1.66	0.46	1.44	0.25	1.50	1.37	0.88	0.51
At4g23650	D7G01	2.36	0.41	2.00	0.10	1.16	0.20	1.37	0.47	1.65	0.44	1.67	0.12
At1g23310	D7G02	1.19	0.22	0.90	0.25	1.86	0.42	1.70	1.12	1.52	0.38	0.6	0.05
At4g35770	D7G03	1.06	0.11	0.86	0.13	1.38	0.13	1.87	0.54	0.89	0.68	1.42	0.12
At5g14780	D7G04	1.48	0.42	1.22	0.26	1.77	0.20	2.25	0.70	1.50	0.50	1.54	0.57
At5g19990	D7G05	0.60	0.01	0.53	0.09	2.08	1.10	1.61	0.72	1.28	2.02	0.72	0.07
At4g02340	D7G06	0.85	0.05	0.73	0.02	2.44	0.94	1.80	0.67	3.30	3.21	1.02	0.08
At1g66140	D7G07	1.24	0.55	1.05	0.31	1.41	0.32	0.95	0.17	0.79	0.35	1.61	0.06
At2g18280	D7G08	1.16	0.25	1.07	0.05	1.37	0.28	1.39	0.69	1.30	0.27	1.1	0.08
At3g13750	P0A01	1.71	0.32	1.59	0.19	1.13	0.97	1.33	0.40	1.17	0.19	1.02	0.04
At2g47580	P0A02	1.68	0.44	1.41	0.29	1.65	0.40	0.97	0.12	1.06	0.25	1.22	0.16
At2g39730	P0A03	1.26	0.26	1.31	0.28	2.09	0.48	2.11	1.33	1.62	1.03	0.85	0.01
At5g38430	P0A04	1.64	0.11	1.24	0.20	1.43	0.04	1.18	0.47	1.26	0.36	0.87	0.07
At3g29030	P0A05	1.16	0.19	1.39	0.14	0.98	0.10	1.09	0.45	1.06	0.29	0.95	0.08
At2g29330	P0A06	1.47	0.29	1.20	0.24	1.10	0.07	1.42	0.44	1.35	0.24	1.04	0.05
At5g38470	P0A07	1.65	0.18	1.59	0.20	1.53	0.25	1.22	0.35	0.93	0.30	1.06	0.12
At1g76890	P0A08	1.39	0.46	1.17	0.22	1.79	0.38	0.94	0.16	1.01	0.33	1.64	0.19
At5g55920	P0A09	1.41	0.36	1.35	0.29	1.62	0.55	1.67	0.18	1.34	0.44	1.49	0.09
At5g61820	P0A10	2.61	0.72	2.52	0.48	4.59	2.80	2.89	0.25	1.51	0.62	1.66	0.3
At1g64230	P0A11	1.67	0.09	1.80	0.30	1.19	0.10	1.31	0.55	1.72	0.36	1.15	0.05
At5g38410	P0A12	1.84	0.43	1.33	0.39	1.37	0.05	1.22	0.31	1.38	0.47	1.26	0.83
At5g08670	P0B01	1.30	0.25	1.23	0.21	1.88	0.43	1.88	0.69	1.44	0.77	0.97	0.06
At2g14100	P0B02	1.65	0.18	1.70	0.27	2.28	0.86	2.94	0.90	1.68	0.67	1.12	0.07
At5g49660	P0B03	1.56	0.07	1.59	0.21	1.80	0.24	1.31	0.18	1.25	0.30	1.01	0.09
At4g23220	P0B04	1.12	0.18	1.36	0.17	1.50	0.20	1.51	0.43	1.30	0.41	1.14	0.31
At1g77990	P0B05	1.51	0.15	1.36	0.29	1.66	0.36	0.97	0.10	0.99	0.31	1.65	0.07
At1g23130	P0B06	1.40	0.16	1.52	0.23	1.57	0.19	1.58	0.70	1.34	0.25	1.33	0.08
At2g21660	P0B07	1.45	0.28	1.46	0.61	1.32	0.26	1.98	0.79	1.41	0.29	1.18	0.3
At2g04780	P0B08	1.61	0.06	1.17	0.26	1.41	0.20	1.33	0.47	1.59	0.89	1.24	0.12

At2g21170	P0B09	1.59	0.05	1.27	0.22	1.87	0.26	1.06	0.15	1.03	0.28	1.43	0.14
At1g73740	P0B10	1.36	0.19	1.20	0.26	1.70	0.39	0.85	0.20	1.02	0.21	1.5	0.13
At4g18360	P0B11	1.34	0.15	1.01	0.23	1.61	0.29	0.98	0.30	1.22	0.25	1.02	0.1
At1g54270	P0B12	1.73	0.08	1.56	0.27	1.75	0.09	1.21	0.09	1.31	0.37	1.29	0.48
At2g36460	P0C01	1.26	0.19	1.45	0.17	1.56	0.10	1.50	0.46	1.09	0.44	1.47	0.07
At1g24180	P0C02	1.68	0.31	1.54	0.38	1.76	0.30	1.08	0.07	1.10	0.20	1.49	0.06
At1g73880	P0C03	1.46	0.12	1.45	0.16	1.33	0.08	1.05	0.33	1.41	0.24	1.62	0.09
At5g65050	P0C04	1.65	0.28	1.58	0.30	1.76	0.37	1.12	0.13	1.33	0.34	1.46	0.06
At3g08770	P0C05	1.46	0.26	1.70	0.53	1.35	0.34	1.23	0.34	2.39	1.96	1.33	0.09
At5g16470	P0C06	1.98	0.50	1.61	0.24	1.22	0.11	1.65	0.41	1.63	0.26	1.4	0.08
At1g06680	P0C07	1.25	0.11	1.20	0.09	1.45	0.17	1.13	0.48	1.57	0.52	1.17	0.12
At3g55120	P0C08	1.43	0.20	1.15	0.14	1.60	0.31	1.05	0.30	1.22	0.43	1.1	0.2
At1g65980	P0C09	1.44	0.10	1.58	0.22	1.37	0.14	1.46	0.55	1.49	0.43	1.2	0.16
At1g09210	P0C10	1.46	0.23	1.44	0.35	1.88	0.37	1.59	0.19	1.06	0.15	0.93	0.16
At5g09660	P0C11	1.39	0.06	1.26	0.02	1.49	0.23	1.34	0.78	1.20	0.18	0.86	0.15
At3g15000	P0C12	1.62	0.23	1.80	0.28	1.50	0.15	1.35	0.04	1.27	0.32	1.21	0.17
At1g79550	P0D01	1.37	0.08	1.30	0.23	1.70	0.30	1.02	0.09	0.97	0.35	1.67	0.36
At5g45710	P0D02	1.61	0.25	1.32	0.31	0.64	1.60	0.97	0.12	1.08	0.25	1.65	0.06
At2g45180	P0D03	1.74	0.14	2.29	0.54	1.28	0.20	1.21	0.37	1.45	0.39	1.53	0.18
At5g46110	P0D04	1.27	0.06	1.27	0.06	1.13	0.11	0.92	0.35	1.30	0.38	1.28	0.04
At5g40580	P0D05	1.37	0.15	1.38	0.28	1.61	0.27	1.02	0.13	1.31	0.28	1.27	0.21
At1g73330	P0D06	1.04	0.67	1.24	0.40	1.86	1.23	1.81	0.47	4.36	5.66	1.28	0.02
At5g10960	P0D07	1.73	0.07	1.69	0.38	1.48	0.11	1.11	0.15	1.40	0.31	1.22	0.06
At2g21660	P0D08	1.58	0.10	1.53	0.27	1.51	0.54	1.63	0.47	1.31	0.30	1.37	0.26
At5g54640	P0D09	1.52	0.31	1.27	0.17	1.51	0.05	1.42	0.16	1.50	0.20	1.26	0.15
At3g48990	P0D10	1.84	0.40	1.54	0.33	1.71	0.38	1.55	0.58	1.63	0.72	1.75	0.98
At5g48760	P0D11	1.56	0.06	1.62	0.18	1.36	0.11	1.03	0.06	1.14	0.27	1.25	0.38
At4g14130	P0D12	2.26	0.08	1.42	0.05	1.48	0.28	0.93	0.12	1.02	0.38	1.62	0.4
At3g09440	P0E01	1.67	0.17	2.78	0.62	2.16	0.30	1.93	0.36	1.28	0.75	1.52	0.05
At4g31720	P0E02	1.63	0.17	1.71	0.25	1.41	0.12	1.40	0.44	1.32	0.35	1.3	0.08
At1g75330	P0E03	1.46	0.11	1.49	0.23	1.53	0.08	1.14	0.10	1.23	0.21	1.42	0.05
At5g20700	P0E04	1.17	0.07	1.58	0.34	1.38	0.19	1.14	0.20	1.37	0.20	1.35	0.06
At1g67090	P0E05	1.62	0.08	1.35	0.38	1.19	0.21	1.09	0.39	1.27	0.32	1.54	0.42
At1g22300	P0E06	1.69	0.16	1.59	0.25	1.54	0.12	1.25	0.33	1.40	0.21	1.22	0.19
At1g05850	P0E07	1.64	0.01	1.35	0.04	1.47	0.17	1.26	0.46	1.40	0.32	1.23	0.07
At2g18110	P0E08	1.75	0.11	1.81	0.26	1.40	0.10	1.12	0.05	1.36	0.22	1.19	0.14
At5g20250	P0E09	2.04	0.63	1.74	0.19	1.96	0.30	1.14	0.25	1.35	0.17	1.14	0.15
At1g04100	P0E10	1.62	0.42	1.51	0.30	0.37	1.95	1.23	0.10	1.52	0.67	1.11	0.21
At5g03240	P0E11	2.31	0.20	2.09	0.19	1.57	0.44	1.98	0.86	1.40	0.31	1.08	0.25
At3g15210	P0E12	3.74	0.59	2.04	0.32	3.11	1.22	1.83	0.61	3.24	2.40	1.38	0.27
At3g56240	P1A01	1.49	0.25	1.55	0.20	1.18	0.04	1.22	0.53	1.27	0.53	0.92	0.07
At5g52310	P1A02	1.58	0.57	1.46	0.44	2.67	0.99	2.96	0.33	1.05	0.62	1.48	0.04
At5g44420	P1A03	2.17	1.10	1.08	0.41	1.50	0.28	2.52	1.40	0.93	0.98	1.55	0.18
At4g23300	P1A04	1.54	0.17	0.97	0.12	1.67	0.47	1.07	0.30	0.87	0.17	0.89	0.1
At1g30510	P1A05	1.29	0.18	1.42	0.44	1.23	0.15	0.88	0.35	1.01	0.39	1.73	0.22
At2g34430	P1A06	1.33	0.16	1.56	0.52	1.31	0.15	0.93	0.28	1.42	0.29	0.99	0.05
At1g05010	P1A07	2.03	0.48	2.48	0.59	1.60	0.17	1.13	0.37	1.33	0.13	1.46	0.36
At4g22330	P1A08	1.66	0.73	1.59	0.66	2.11	0.48	1.43	0.25	1.70	0.28	1.25	0.16
At2g40940	P1A09	1.50	0.64	1.51	0.93	3.15	1.56	1.87	0.75	1.19	0.54	0.87	0.09
At1g12270	P1A10	1.82	0.54	1.37	0.59	1.82	0.16	1.66	0.30	1.18	0.33	1.24	0.11
At1g31580	P1A11	1.59	0.11	1.81	0.27	1.30	0.13	1.08	0.36	1.07	0.48	1.75	0.1
At4g02520	P1A12	1.84	0.85	1.09	0.28	1.38	0.20	1.15	0.30	1.23	0.36	0.73	0.06
At5g13930	P1B01	1.32	0.13	1.52	0.60	1.24	0.14	0.95	0.35	1.44	0.22	1.01	0.11

At2g34420	P1B02	1.41	0.06	1.45	0.54	1.21	0.17	0.95	0.27	1.39	0.30	1	0.01
At1g77760	P1B03	2.61	0.51	1.77	0.14	1.47	0.13	1.04	0.32	1.29	0.19	1.15	0.07
At3g55430	P1B04	3.24	0.42	2.57	0.98	2.58	0.59	1.72	0.50	1.50	0.37	2	0.17
At1g07890	P1B05	2.97	0.41	3.67	1.35	2.58	0.64	1.99	0.66	1.82	0.38	1.6	0.09
At3g22370	P1B06	2.55	0.06	1.79	0.50	2.56	0.24	1.03	0.16	0.98	0.42	2.61	0.41
At3g01420	P1B07	1.45	0.39	1.03	0.22	2.54	0.34	1.29	0.16	1.78	0.73	1.82	0.19
At5g20230	P1B08	19.9	10.5	8.71	0.73	17.6	11.2	3.62	1.23	12.2	4.54	5.17	0.71
At3g49120	P1B09	2.66	0.06	1.99	0.46	2.47	0.50	2.01	0.13	1.98	0.58	2.05	0.17
At3g12500	P1B10	1.81	0.73	1.47	0.20	1.83	0.20	1.25	0.28	1.48	1.15	1.6	0.19
At2g31570	P1B11	1.47	0.23	1.98	0.09	1.43	0.15	1.16	0.18	1.04	0.38	2.05	0.18
At3g24170	P1B12	1.45	0.10	1.66	0.52	1.86	0.13	2.18	0.50	0.74	1.43	0.78	0.06
At2g34430	P1C02	1.61	0.18	1.62	0.04	1.42	0.17	1.02	0.12	1.30	0.35	1.21	0.09
At3g10920	P1C03	4.15	0.07	3.54	1.62	3.03	1.22	2.10	0.49	2.24	0.93	1.42	0.07
At5g42650	P1C04	4.81	0.61	5.81	1.71	3.64	1.79	2.51	0.34	2.97	2.06	1.79	0.1
At1g20630	P1C05	1.58	0.15	1.38	0.39	1.99	0.40	1.87	0.67	2.09	0.59	1.51	0.08
At5g40370	P1C06	2.33	0.35	2.26	0.54	1.79	0.39	1.54	0.46	1.84	0.53	1.59	0.11
At1g62180	P1C07	1.79	0.46	1.44	0.30	1.74	0.22	1.12	0.32	1.31	0.42	1.88	0.16
At4g16520	P1C08	1.35	0.16	1.53	0.25	1.38	0.21	0.84	0.11	0.88	0.44	2.42	0.24
At1g21270	P1C09	1.54	0.34	1.36	0.58	2.16	0.32	1.46	0.43	2.85	0.76	2.08	0.26
At4g11600	P1C10	2.05	0.31	2.14	0.77	2.54	1.23	1.81	0.61	1.96	0.34	1.52	0.1
At1g78300	P1C11	1.57	0.39	1.73	0.15	1.47	0.27	1.29	0.40	1.48	0.25	1.08	0.08
At3g21510	P1C12	1.70	0.49	1.39	0.23	1.45	0.14	1.24	0.27	1.23	0.37	1.71	0.22
At3g29350	P1D01	1.46	0.36	1.47	0.35	1.33	0.20	1.20	0.42	1.07	0.44	0.92	0.06
At5g39340	P1D02	1.47	0.32	1.36	0.33	1.26	0.11	1.06	0.28	1.32	0.37	0.91	0.06
At1g66340	P1D03	1.70	0.54	1.32	0.30	2.05	0.50	1.52	0.33	1.68	0.33	1.44	0.09
At3g04580	P1D04	1.85	0.83	1.28	0.54	2.40	0.57	1.63	0.31	1.61	0.33	1.41	0.09
At2g01830	P1D05	1.61	0.67	0.94	0.52	3.77	0.97	1.75	0.46	2.75	1.70	1.35	0.07
At3g16857	P1D06	1.37	0.41	1.53	1.09	1.92	0.38	1.69	0.52	1.78	0.48	1.31	0.06
At1g10470	P1D07	1.89	0.40	1.42	0.32	1.66	0.18	1.03	0.30	1.81	0.52	1.28	0.07
At3g48100	P1D08	1.89	0.55	0.90	0.32	1.34	0.15	0.83	0.21	1.63	0.34	1.23	0.07
At5g62920	P1D09	2.09	0.49	1.10	0.32	1.36	0.17	0.90	0.24	1.77	0.33	1.18	0.08
At1g72610	P1D10	1.65	0.31	1.31	0.13	1.18	0.17	0.96	0.28	1.47	0.48	0.86	0.04
At5g20630	P1D11	1.24	0.17	0.95	0.38	0.98	0.27	0.66	0.18	1.70	0.73	0.36	0.02
At1g18970	P1D12	1.39	1.31	1.03	0.38	0.63	1.92	1.91	1.08	1.85	0.72	0.84	0.06
At1g09560	P1E01	2.15	0.63	1.67	0.55	1.89	0.42	1.05	0.15	1.41	1.10	1.1	0.04
At5g39100	P1E02	-2.7	6.76	0.50	0.63	1.48	0.45	1.18	0.33	0.47	1.79	0.97	0.07
At1g10460	P1E03	1.50	0.43	1.22	0.57	1.93	0.52	1.32	0.29	1.58	0.36	1.34	0.05
At3g05930	P1E04	1.79	0.69	1.30	0.50	1.62	0.27	1.35	0.32	1.51	0.22	1.33	0.06
At3g14630	P1E05	1.61	0.64	1.26	0.45	1.82	0.50	1.27	0.35	1.57	0.36	1.31	0.07
At1g08500	P1E06	0.49	1.09	0.40	0.55	1.40	0.24	1.28	0.49	1.40	0.22	1.29	0.05
At3g23150	P1E07	1.45	0.56	4.99	6.83	1.50	0.26	1.07	0.28	1.73	0.50	1.22	0.05
At4g39330	P1E08	0.89	0.15	1.26	0.17	1.11	0.26	1.02	0.29	1.30	0.23	1.2	0.06
At1g67980	P1E09	1.43	0.26	1.34	0.43	1.24	0.19	0.94	0.26	1.42	0.20	1.2	0.07
At5g54160	P1E10	1.59	0.15	1.92	0.30	1.38	0.12	1.10	0.30	1.47	0.34	0.98	0.05
At1g69370	P1E11	3.25	1.43	1.74	0.71	1.65	0.41	1.13	0.30	1.66	0.69	0.6	0.04
At4g31500	P1E12	4.36	0.22	3.26	0.71	3.25	0.71	3.27	0.98	5.08	3.88	3.75	0.42
At3g53260	P1F01	1.30	0.46	1.41	0.63	1.49	0.12	1.48	0.76	1.48	0.74	1.9	0.15
At5g04230	P1F02	3.22	3.22	1.08	0.22	1.91	0.37	1.59	0.44	1.88	0.91	1.57	0.12
At2g37040	P1F03	1.50	0.29	1.31	0.11	1.57	0.14	1.33	0.39	1.53	0.10	1.22	0.04
At1g51680	P1F04	1.55	0.39	1.31	0.14	1.60	0.09	1.37	0.39	1.62	0.22	1.3	0.06
At1g55020	P1F05	1.89	0.60	2.17	1.09	4.11	2.19	3.77	1.03	1.76	0.43	1.26	0.09
At3g45140	P1F06	2.58	0.62	2.75	0.93	3.70	2.11	3.18	1.07	1.68	0.77	1.26	0.06
At4g08500	P1F07	4.91	4.17	1.77	1.13	2.41	0.42	2.04	0.43	1.53	0.29	1.21	0.06

At3g45640	P1F08	2.88	0.44	1.36	0.40	1.85	0.71	1.90	0.82	1.12	0.83	1.15	0.05
At3g56860	P1F09	1.61	0.63	1.34	0.35	1.60	0.20	1.24	0.33	1.48	0.26	1.14	0.05
At4g38530	P1F10	1.65	0.65	1.18	0.67	1.55	0.33	1.09	0.31	4.43	6.22	0.97	0.05
At3g47290	P1F11	0.62	1.58	1.41	0.54	1.33	0.11	1.06	0.38	1.50	0.29	0.94	0.08
At2g34420	P1F12	1.32	0.14	1.56	0.59	1.31	0.16	1.04	0.36	1.42	0.36	1.03	0.05
At5g61590	P1G01	2.62	1.26	1.60	0.15	2.06	0.50	1.56	0.39	1.25	0.83	0.81	0.03
At1g67710	P1G02	1.85	0.85	1.18	0.49	1.75	0.24	1.33	0.39	1.60	0.25	1.13	0.07
At4g16110	P1G03	2.41	1.34	0.98	0.44	2.03	0.30	1.53	0.41	1.96	0.36	1.47	0.1
At1g64280	P1G05	3.19	1.07	1.48	0.81	2.78	0.70	1.58	0.56	2.35	1.20	1.17	0.06
At3g20600	P1G06	3.53	1.15	1.45	0.61	3.13	1.10	1.54	0.31	2.95	2.28	1.11	0.07
At1g62380	P1G07	1.51	0.53	11.0	16.1	1.83	0.35	1.96	0.56	2.01	0.82	1.12	0.1
At3g61510	P1G08	1.88	0.79	0.14	2.08	2.14	0.38	1.63	0.48	1.75	0.39	0.89	0.05
At1g01480	P1G09	1.58	3.51	1.29	3.19	2.00	2.43	0.80	3.86	2.24	2.14	0.61	0.07
At2g22810	P1G10	1.88	1.21	0.44	1.93	2.26	1.31	1.45	0.40	1.04	0.71	0.66	0.07
At5g65800	P1G11	0.34	0.19	0.45	0.57	1.23	0.16	1.03	0.45	1.53	0.37	0.92	0.06
At4g11280	P1G12	1.26	5.08	0.50	0.61	1.24	0.17	1.01	0.40	1.46	0.31	0.93	0.09
At1g27320	P1H01	1.71	0.23	1.27	0.14	1.84	0.34	1.37	0.40	1.52	0.44	1.12	0.06
At3g15210	P1H02	5.59	1.40	2.48	0.73	2.82	0.69	1.42	0.23	2.52	1.06	2.14	0.06
At2g40940	P1H03	1.00	0.16	2.06	1.98	2.61	0.78	1.51	0.45	2.26	0.69	1.92	0.02
At1g04310	P1H04	3.07	2.46	0.96	0.67	5.95	4.15	1.69	1.03	1.58	0.56	1.46	0.08
At1g59870	P1H05	6.43	6.36	0.93	0.55	10.8	7.59	1.85	0.97	0.08	2.34	0.47	0.04
At1g30900	P1H06	1.14	0.53	0.99	0.65	3.97	1.98	1.46	0.38	0.25	1.21	0.81	0.07
At3g60820	P1H07	1.52	0.37	1.42	0.29	0.98	1.64	0.46	0.49	0.75	1.37	0.91	0.06
At3g16450	P1H08	1.57	0.48	1.23	0.19	2.44	0.82	2.78	0.74	0.79	0.75	0.6	0.11
At2g32340	P1H09	1.56	0.59	1.23	0.36	1.64	0.52	1.79	0.32	0.57	0.75	0.79	0.11
At4g12480	P1H10	1.32	0.02	2.36	0.59	2.02	0.76	2.16	0.51	3.27	2.38	1.49	0.16
At4g30190	P1H11	1.92	1.10	0.07	2.18	2.13	0.26	2.17	1.03	0.05	0.49	0.69	0.09
At3g53990	P1H12	1.99	0.43	1.48	0.20	1.50	0.13	1.19	0.27	1.87	0.18	1.17	0.06
At5g48380	P2A01	1.21	0.66	0.87	0.69	3.10	1.30	1.33	0.78	0.31	0.50	0.39	0.09
At3g27380	P2A02	1.20	0.14	0.85	0.38	1.58	0.34	1.40	0.46	1.19	0.66	1.36	0.09
At1g27450	P2A03	1.23	0.45	1.13	0.35	1.42	0.16	1.35	0.39	1.40	0.89	1.03	0.07
At2g28190	P2A04	6.91	10.1	0.73	0.32	1.41	0.25	1.44	0.48	1.53	0.68	0.64	0.11
At1g10760	P2A05	0.93	0.26	0.98	0.59	1.58	0.13	1.59	0.55	1.52	1.03	0.44	0.01
At4g30650	P2A06	1.00	0.35	0.85	0.21	1.58	0.59	1.82	0.28	0.60	1.50	0.97	0.09
At1g64720	P2A07	0.51	1.84	1.32	0.87	1.16	0.45	0.96	0.73	1.21	0.47	0.55	0.04
At3g26520	P2A08	1.97	0.24	2.24	0.15	1.43	0.26	1.46	0.50	2.09	1.20	1.29	0.04
At1g07610	P2A09	1.97	0.31	2.38	0.60	1.51	0.73	1.65	0.05	1.64	0.46	0.85	0.02
At2g24180	P2A10	1.39	0.22	1.37	0.38	3.99	4.81	1.71	0.51	2.14	2.35	0.87	0.07
At3g48090	P2A11	1.81	0.42	1.61	0.10	1.79	0.36	1.83	0.15	0.68	1.26	0.67	0.09
At3g16470	P2A12	1.77	0.49	3.71	1.85	3.09	1.38	5.71	1.47	1.71	0.57	7.88	1.3
At3g22370	P2B01	1.81	0.46	1.56	0.74	2.52	0.03	1.46	0.68	0.85	0.84	1.34	0.03
At4g20860	P2B02	0.36	1.57	1.53	0.98	1.74	0.16	1.63	0.76	1.12	0.28	1.02	0.13
At1g20620	P2B03	1.48	0.53	0.85	0.14	1.61	0.38	1.97	1.04	1.50	0.78	0.5	0.03
At1g08830	P2B04	1.33	0.14	1.36	0.24	1.37	0.12	1.36	0.49	1.57	0.11	1.17	0.05
At4g25100	P2B05	1.05	0.38	0.87	0.46	1.37	0.16	1.32	0.60	0.71	1.45	0.74	0.04
At4g04610	P2B06	1.75	0.27	1.16	0.34	2.13	0.14	1.27	0.41	1.55	0.30	1.61	0.09
At2g25080	P2B07	1.42	0.10	1.38	0.19	1.26	0.09	1.09	0.48	1.29	0.49	0.8	0.05
At1g02920	P2B08	4.85	0.52	5.03	1.86	3.31	0.32	3.14	1.14	3.11	3.10	2.7	0.19
At2g34660	P2B09	1.65	0.31	1.31	0.37	2.96	1.29	2.15	0.31	0.84	1.05	1.34	0.08
At1g02930	P2B10	2.73	0.78	3.11	1.19	2.96	0.46	2.22	0.83	2.73	2.78	4.21	0.41
At4g00860	P2B11	1.69	0.16	1.70	0.35	1.50	0.20	1.39	0.30	1.60	0.32	1.2	0.03
At2g14610	P2B12	1.50	0.25	3.16	2.49	2.23	2.12	2.21	0.32	4.02	4.81	9.23	0.98
At3g57260	P2C01	1.74	0.32	2.11	1.34	1.57	0.16	1.57	0.81	1.92	2.00	6.43	0.4

At1g75040	P2C02	1.43	0.25	2.01	1.43	1.37	0.21	1.07	0.45	1.81	1.86	2.83	0.19
At3g57260	P2C03	2.00	1.05	1.64	1.78	1.20	0.11	0.97	0.41	1.40	0.51	1.56	0.06
At3g57240	P2C04	1.70	0.77	1.93	1.84	1.59	0.06	1.30	0.45	1.58	0.27	1.65	0.25
At5g24090	P2C05	1.12	0.37	0.68	0.26	2.98	2.13	1.42	0.27	1.27	0.43	1.45	0.06
At3g54420	P2C06	1.58	0.73	1.46	0.63	2.40	1.70	1.67	0.73	2.01	0.84	1.36	0.03
At2g20340	P2C07	1.45	0.35	1.30	0.24	1.39	0.11	1.17	0.48	1.61	0.24	1.33	0.06
At4g36430	P2C08	0.96	0.56	0.59	0.45	1.64	0.16	1.25	0.50	1.68	0.47	1.22	0.06
At5g06870	P2C10	5.02	1.39	3.69	1.24	4.26	1.37	3.03	0.52	3.05	1.84	3.59	0.26
At2g14890	P2C11	1.33	0.29	1.14	0.16	1.68	0.03	1.17	0.57	1.80	0.86	0.64	0.05
At3g07010	P2C12	1.13	0.22	1.02	0.15	1.08	0.31	0.83	0.50	0.88	0.44	0.3	0.05
At1g53840	P2D01	1.51	0.70	0.80	0.37	2.09	0.44	1.54	0.67	2.36	2.39	1.03	0.23
At2g23600	P2D02	1.25	0.27	1.57	0.27	1.47	0.09	1.61	0.53	1.27	0.29	1.15	0.08
At2g39940	P2D03	1.86	0.22	1.39	0.60	1.65	0.12	1.94	0.70	1.58	0.44	1.36	0.07
At3g28940	P2D04	1.98	0.27	1.79	0.73	1.43	0.05	1.32	0.45	1.54	0.21	1.37	0.05
At2g35980	P2D05	1.60	0.37	1.16	0.36	1.97	0.38	1.55	0.35	2.12	0.76	1.37	0.08
At1g72260	P2D06	1.17	0.57	1.21	0.57	1.90	0.56	1.48	0.36	1.94	0.66	1.25	0.09
At4g30280	P2D07	7.64	3.50	1.51	0.41	2.59	0.55	1.39	0.27	3.03	1.87	1.29	0.08
At5g47550	P2D08	1.79	0.41	1.26	0.51	1.61	0.06	1.36	0.24	1.67	0.30	1.26	0.07
At2g40880	P2D09	1.45	0.24	2.61	0.55	1.49	0.04	1.56	0.30	1.67	0.31	1.31	0.13
At2g02100	P2D10	1.35	0.18	1.71	0.36	1.56	0.02	1.69	0.34	2.37	0.65	1.28	0.08
At3g16420	P2D11	2.13	1.01	2.37	1.08	2.04	0.84	4.59	1.94	0.04	2.79	1.76	0.17
At5g65790	P2D12	1.65	0.98	0.53	0.75	1.15	4.23	2.51	1.39	1.37	0.41	1.47	0.1
At2g45820	P2E01	1.88	0.38	1.21	0.26	1.51	0.12	1.07	0.34	1.29	0.38	1.12	0.05
At5g16050	P2E02	1.47	0.09	2.19	0.33	1.42	0.19	1.90	0.63	1.20	0.16	1.04	0.08
At3g44320	P2E03	1.38	0.14	3.43	0.49	1.31	0.07	2.29	0.73	1.38	0.37	1.68	0.08
At3g20130	P2E04	1.17	0.33	1.01	0.17	1.96	0.14	1.02	0.12	1.14	0.13	1.65	0.08
At5g13110	P2E05	1.17	0.32	1.07	0.43	1.67	0.30	1.67	0.43	1.40	0.23	1.36	0.07
At3g25230	P2E06	1.65	0.16	1.53	0.38	1.53	0.09	1.21	0.20	1.36	0.23	1.3	0.07
At1g67980	P2E07	1.37	0.21	1.29	0.39	1.63	0.06	1.36	0.34	1.83	0.46	1.27	0.07
At1g23760	P2E09	1.49	0.53	0.96	0.31	1.59	0.11	1.54	0.31	1.75	0.53	1.22	0.05
At3g12120	P2E10	1.52	0.10	1.30	0.26	1.54	0.18	1.55	0.55	1.76	0.51	1.02	0.03
At3g55120	P2E11	1.31	0.26	1.26	0.42	1.61	0.29	0.89	0.13	0.95	0.41	1.78	0.14
At5g08640	P2E12	1.13	0.39	1.01	0.43	1.84	0.53	1.76	0.36	1.07	0.72	0.74	0.03
At1g75280	P2F01	1.82	0.24	1.34	0.37	2.65	0.46	1.93	1.05	1.63	0.46	0.94	0.05
At3g51240	P2F02	0.93	0.23	1.20	0.23	1.70	0.02	2.32	0.81	0.79	0.87	0.55	0.08
At3g04720	P2F03	4.51	0.81	3.44	1.95	1.80	0.26	2.57	0.94	2.18	2.15	1.52	0.1
At1g02930	P2F04	5.16	0.68	4.79	2.01	7.51	4.49	4.28	1.99	3.20	3.95	2.2	1.51
At2g29420	P2F05	5.30	2.15	2.30	1.04	11.2	10.6	2.98	0.99	3.88	2.99	1.26	0.04
At1g77490	P2F06	1.16	0.36	1.24	0.18	1.43	0.04	1.18	0.48	1.50	0.34	1.22	0.08
At5g24770	P2F07	1.78	0.75	2.03	0.93	4.36	1.62	4.66	2.29	0.76	0.89	1.26	0.15
At4g37980	P2F08	1.66	0.35	1.44	0.18	1.54	0.13	1.48	0.46	1.56	0.52	0.98	0.07
At5g05730	P2F09	1.68	0.62	1.57	0.55	4.47	1.69	4.71	2.26	1.83	1.24	1.09	0.13
At1g25165	P2F10	4.13	1.25	2.50	1.07	3.12	0.88	2.70	0.95	2.93	1.44	1.92	0.18
At3g54640	P2F11	2.06	0.39	2.93	0.94	1.72	0.25	0.96	0.27	0.96	0.43	2.37	0.06
At5g54810	P2F12	1.69	0.35	9.06	13.2	2.77	1.06	2.71	1.04	2.42	0.59	2.06	0.11
At5g07010	P2G01	1.38	0.20	2.09	0.76	2.18	0.58	1.81	0.54	1.82	0.49	1.66	0.09
At3g11170	P2G02	3.15	0.14	2.59	1.12	3.98	1.93	2.51	1.07	2.38	0.95	0.77	0.06
At5g42650	P2G03	3.79	0.57	4.20	2.29	5.66	3.38	3.53	1.38	6.74	2.73	11.9	1.31
At1g21250	P2G04	2.22	0.23	1.78	0.78	3.21	0.74	0.81	1.10	2.19	0.88	1.99	0.21
At3g20600	P2G05	3.52	1.17	1.38	0.54	2.03	0.41	1.29	0.46	2.21	0.83	1.42	0.14
At4g01150	P2G06	1.14	0.25	1.11	0.11	1.28	0.04	1.11	0.42	1.46	0.20	1.08	0.07
At3g25230	P2G07	1.53	0.29	1.33	0.22	1.48	0.15	1.33	0.42	1.50	0.19	1.14	0.1
At4g01850	P2G08	2.19	0.21	1.64	0.34	2.57	0.48	2.35	0.94	2.03	0.74	1.03	0.11

At3g23810	P2G09	1.50	0.16	2.65	0.11	1.87	0.38	1.93	0.39	1.90	0.79	1.74	0.16
At5g56650	P2G10	1.91	0.66	1.36	0.20	2.39	1.02	1.32	0.33	1.35	1.25	1.38	0.19
At5g24780	P2G11	2.02	1.07	2.55	1.53	4.65	1.59	4.86	2.34	0.89	0.90	9.18	2.14
At1g04400	P2G12	0.98	0.21	1.32	0.64	1.74	0.19	1.92	0.82	1.90	1.80	1.06	0.09
Control	P2H01	1.19	0.44	0.82	0.33	0.32	0.20	0.37	0.62	0.72	1.22	0.63	0.11
Control	P2H02	1.09	0.31	1.03	0.37	1.45	0.29	1.45	0.86	3.11	8.14	0.24	0.07
Control	P2H03	1.09	0.43	1.39	1.04	1.69	0.28	1.52	0.72	1.46	1.11	1.34	0.15
At5g41150	P2H04	1.33	0.71	2.53	3.16	1.67	0.12	1.35	0.51	1.41	0.29	1.3	0.06
At1g12370	P2H05	1.25	0.67	0.68	0.36	1.62	0.15	1.35	0.51	1.49	0.39	1.27	0.27
At4g10490	P2H06	9.89	18.6	0.99	0.72	1.44	0.01	1.18	0.43	1.56	0.18	1.5	0.15
At3g15620	P2H07	1.41	0.49	0.90	0.32	1.51	0.18	1.36	0.51	1.48	0.27	1.42	0.21
At1g09100	P2H09	1.31	0.58	0.96	0.54	0.91	2.58	1.09	1.41	0.12	0.50	0.81	0.16
At4g15480	P2H10	1.10	0.47	0.98	0.45	1.07	0.35	2.10	0.36	5.16	12.5	0.95	0.06
At4g15490	P2H11	1.33	0.51	1.45	0.97	0.94	9.42	3.96	1.35	0.61	1.90	1.49	0.16
At4g15500	P2H12	1.33	0.41	1.10	0.28	2.48	0.95	2.42	0.55	1.08	0.82	1.5	0.19

The genes with weak signals (lower than 2 fold local background) were neglected by grey scripts. The genes with induction over 3.5 fold are marked by red colored boxes, the ratio below 0.5 are marked with green boxes, and with induction between 2.5-3.5 fold by orange colored boxes. Array data were based on four independent replicates and the standard deviation (SD) were shown on the right side of each ratio.

Acknowledgements

I am most grateful to my supervisor PD Dr. J. Durner for his guidance, support and confidence during this work, and I thank him for his suggestions, excellent correction and accurate review of this the manuscripts.

I express my profound gratitude and deep appreciation to Prof. Dr. H. Sandermann for accepting me as a member of BIOP and fruitful discussion of my work.

I would like to express my sincere gratitude to Prof. Dr. E. Grill for accepting to be my referee.

I sincerely thank Prof. Dr. E. F. Elstner for accepting me for examination.

My profound gratitude goes to Dr. C. Lindermayer and Dr. U. von Rad for his fruitful discussion and the correction of this manuscript.

I am also grateful to E. Mattes and G. Fricke-Bode for technical assistance during the period of this work.

I sincerely thank the staffs of work group of plant immunity for their friendly collaboration and assistance.

My thanks go to the staffs of BIOP for their help in one or other way during the period of this work.

I wish to express my gratitude to R. Ludwig, W. Mayr for ethylene and salicylic acid determination.

My cordial thanks to K. Stettmaier for assistance with EPR and Dr. P. Hutzler for help in confocal microscopie.

Sincere thanks go to work group of Dr. M. J. Mueller in University of Wuerzburg for JA determination.

I am indebted to my wife and my children for their morale assistance and patience.

Finally, I thank the “Deutsche Forschungsgemeinschaft (DFG)” for financial support.

Curriculum vitae

Name	Xi Huang
Date of birth	16. 10.1969
Place of birth	Hainan, P. R. China
Parents	Mother Huanying Li and father Tiancheng Huang
1977-1982	Primary school in Qionghai, Hainan
1982-1988	Middle school in Qionghai, Hainan
1988-1992	Study of biology in Peking University
1992	Bachelor
1992-1998	Work in Biotechnical Department in Hainan Academy of Agricultural Sciences
31. 03. 1998	Come to Germany as DAAD scholarship holder
Mar. - Oct. 1998	Learn German in Goethe-Institute in Göttingen
Oct.1998-Oct.2000	Master of science student at the Institute of Plant Pathology and Plant Protection, Georg-August-University, Göttingen
Oct. 2000	Master of Science
Nov. 2000 to date	Ph. D student at Institute of Biochemical Plant Pathology, GSF-National Research Center for Environment and Health, Munich