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S-NITROSOTHIOLS AND REACTIVE OXYGEN SPECIES IN PLANT DISEASE RESISTANCE AND DEVELOPMENT

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Contents

Declaration

I hereby declare that the work presented here is my own and has not been submitted in any form for any degree at this or any other university. Any contribution made by other parties is clearly acknowledged.

Kerstin Brzezek

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Abstract

Nitric oxide (NO) as well as reactive oxygen species (ROS) play an important role in defence signalling in plants. After successful recognition of an invading pathogen, an increase in ROS occurs, the 'oxidative burst'; and a 'nitrosative burst' is also observed. This leads to the induction of defence responses, including the 'hypersensitive response' (HR), a form of programmed cell death. A balanced production of hydrogen peroxide and NO is crucial for HR induction. In a process called S-nitrosylation, NO can react with cysteine thiols to form S-nitrosothiols, or react with glutathione to form S-nitrosoglutathione (GSNO). The enzyme GNSO reductase (GSNOR) indirectly regulates SNO levels by turning over GNSO. The *Arabidopsis thaliana* T-DNA insertion mutant *atgsnor1-3* shows a complete loss of GNSOR activity and has drastically increased SNO levels, resulting in stunted growth, loss of apical dominance, increased HR, loss of salicylic acid (SA) accumulation and increased susceptibility to avirulent, virulent and non-host pathogens. Two recessive and allelic EMS suppressor mutants in the *atgsnor1-3* background were isolated, which showed mostly wild type growth. The mutations were identified by map-based cloning as two different point mutations in *At1g20620* or *CAT3*, one of three catalase genes in Arabidopsis. Catalases break down hydrogen peroxide, with

CAT2 being the major catalase in Arabidopsis. All three catalases are structurally very similar, but show temporal and spatial differences in their expression patterns. The suppressor mutants recovered apical dominance, and partially recovered disease resistance to avirulent pathogens, but were still susceptible to virulent pathogens and showed decreased SA levels. The suppressor mutants showed wild type HR in response to different avirulent bacteria. Interestingly, loss-of-function of the other catalase genes as well as loss-of-function of other redox-related genes did not restore apical dominance of *atgnsor1-3 plants*. This effect seems to be highly specific to *CAT3*, possibly because of its expression pattern or its expression levels. Further research is needed to fully understand the mechanisms at work here, but these results certainly seem to show a direct connection between redox signalling and S-nitrosylation.

Lay Summary

Plant defence signalling in response to a pathogen infection is associated with an increase in nitric oxide (NO) levels as well as an increase in hydrogen peroxide levels, which then leads to the induction of defence responses. One of the defence responses is the 'hypersensitive response' (HR), a localised cell death at the site of infection to stop the pathogen from spreading. In a process called S-nitrosylation, NO binds to certain proteins to form S-nitrosothiols (SNOs). SNO levels are regulated by an enzyme called GSNO reductase (GSNOR). The *Arabidopsis thaliana* (thale cress) mutant *atgsnor1-3* shows a complete loss of GSNOR activity and as a result exhibits drastically increased SNO levels, resulting in stunted and bushy growth, susceptibility to a wide range of plant pathogens, as well as increased HR compared to wild type plants. Two suppressor mutants were identified, which in addition to loss of GSNOR activity, contain a second mutation, which resulted in loss of CAT3 activity. CAT3 is one of three catalases in Arabidopsis. Catalases break down hydrogen peroxide, with CAT2 being the major catalase in Arabidopsis. The three catalases show very similar structures but they occur in different plant tissues and at different times during plant development. The suppressor mutants show wild type growth, and also have partially restored disease resistance to certain

pathogens, as well as showing wild type HR. However, they are still suscepti-
ble to some pathogens and are also still impaired in other aspects of disease
signalling. Interestingly, this effect seems to be highly specific to CAT3, a loss
of function of the other catalases in the *atgsnor1-3* mutant did not restore wild
type growth, possibly due to the different locations of these catalases in the
plant or due to their different levels of activity. This work gives new insights
into plant disease signalling, but further research is needed to fully understand
the mechanisms at work here.

List of Abbreviations

ABA	Abscisic Acid
AOX	Alternative Oxidase
APX	Ascorbate Peroxidase
ASC	Ascorbate
Avr	Avirulence
BAK1	BRI1-Associated Kinase 1
BTP/POZ	Broad-Complex, Tramtrack, and Bric-A-Brac/Poxvirus, Zinc Finger
bud1	*Bushy and Dwarf 1*
CA	Carbonic Anhydrase
CaM	Calmodulin
CAPS	Cleaved Amplified Polymorphic Sequence
CAT	Catalase
CC	Coiled-Coil
CK	Cytokinin
CNGC	Cyclic Nucleotide-Gated Ion Channel
COI1	Coronatine Insensitive 1
Col-0	Columbia-0
CPB60g	CaM-Binding Protein 60g
CTAB	Cetyltrimethylammonium Bromide
CUL	Cullin
Cys	Cysteine
DAB	Diaminobenzidine
DAMP	Damage-Associated Molecular Pattern

DHASC	Oxidised Ascorbate
DND1	Defence, No Death
EAR	ERF-Associated Amphiphilic Repression
EDR1	Enhanced Disease Resistance 1
EDS1	Enhanced Disease Susceptibility 1
EFR	Elongation Factor Receptor
EF-Tu	Elongation Factor - Tu
elf18	18 amino acid fragment of EF-Tu
elf26	26 amino acid fragment of EF-Tu
EMS	Ethylmethane Sulphonate
ERF	Ethylene Response Factor
ET	Ethylene
ETI	Effector-Triggered immunity
ETS	Effector-Triggered susceptibility
flg22	Flagellin22
FLS2	Flagellin-Sensing 2
FMO1	Flavin-Dependent Monooxygenase
GA	Gibberellin
GADPH	Glyceraldehyde 3-Phosphate Dehydrogenase
GR	Glutathione Reductase
GRX	Glutaredoxin
GSH	Glutathione
GSNO	S-Nitrosoglutathione
GSNOR	GSNO Reductase
GSSG	Oxidised Glutathione
H_2O_2	Hydrogen Peroxide
HAMP	Herbivore-Associated Molecular Pattern
hpi	Hours post inoculation
HR	Hypersensitive Response
HSF	Heat Shock Factor
HSP	Heat Shock Protein
ICS1	Isochorismate Synthase 1

InDel	Insertion/Deletion
JA	Jasmonic Acid
JAZ	Jasmonate ZIM
Ler	*Landsberg erecta*
LOL1	LSD1-Like
LPS	Lipopolysaccharide
LRR	Leucine-Rich Repeat
LSD1	Lesions Stimulating Disease 1
MAMP	Microbial-Associated Molecular Pattern
MAPK	Mitogen-Activated Protein Kinase
MAPKKK	Mitogen-Activated Protein Kinase Kinase Kinase
MC9	Metacaspase 9
MeSA	Methyl Salicylate
MV	Methyl Viologen
NB	Nucleotide Binding
NDPK2	Nucleotide Diphosphate Kinase 2
NDR1	Non-Race Specific Disease Resistance Protein 1
NIMIN	NIM-Interacting
NINJA	Novel Interactor of JAZ
NLS	Nuclear Localisation Signal
NO	Nitric Oxide
NOA1	Nitric Oxide Associated 1
NOE1	Nitric Oxide Excess 1
NOS	Nitric Oxide Synthase
NPR1	Non-Expressor of PR Genes
NR	Nitrate Reductase
$O_2^{\bullet-}$	Superoxide
$ONOO^-$	Peroxynitrite
ORA59	Octadecanoid Responsive Arabidopsis 59
PAD4	Phytoalexin Deficient 4
PAMP	Pathogen-associated molecular pattern
PBS1	AvrPphB Susceptible 1
PCD	Programmed Cell Death
PCR	Polymerase Chain Reaction

PDF1.2	Plant Defensin 1.2
PR	Pathogenesis Related
PRR	Pattern-Recognition Receptor
PRX	Peroxiredoxin
Pst	*Pseudomonas syringae* pv *tomato*
PTI	Pattern-triggered immunity
R	Resistance
RBOH	Respiratory Burst Oxidase Homologue
Rf	Recombination Frequency
RIN4	RPM1-Interacting Protein 4
RLK	Receptor-Like Kinase
RLP	Receptor-Like Protein
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
RPM1	Resistance to P. syringae pv tomato 1
RPS2	Resistant to *P. syringae* 2
RPS5	Resistant to *P. syringae* 5
SA	Salicylic Acid
SABP	Salicylic Acid Binding Protein
SAG101	Senescence Associated Gene 101
SAR	Systemic Acquired Resistance
SE	Standard Error
SID2	SA Induction Deficient 2
SNAP	Single Nucleotide Amplied Polymorphism
SNI1	Suppressor of NPR1-Inducible 1
SNO	S-Nitrosothiol
SNP	Single Nucleotide Polymorphism
SOD	Superoxide Dismutase
TAIR	The *Arabidopsis* Information Resource
TIR	Toll Interleukin 1 Receptor
TIR1	Transport Inhibitor Response 1
TRX	Thioredoxin
TTE	Type III Effector
VSP2	Vegetative Storage Protein 2

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Chapter 1

Introduction

1.1 The plant immune system

Even though plants are constantly exposed to pathogens, disease occurs rarely. Plants, unlike mammals, do not possess circulating immune cells and somatic adaptive immunity but they have advanced innate immunity [Fig. 1.1] [Jones and Dangl, 2006]. In order to infect a plant, a pathogen must first access the plant interior by breaching physical barriers like the waxy cuticle and the rigid, cellulose-based cell wall. If a pathogen gets past this first line of defence, it is recognised by transmembrane pattern-recognition receptors (PRRs). PRRs recognise conserved microbial structures, so-called microbial- or pathogen-associated molecular patterns (MAMPs or PAMPs), or damage-associated molecular patterns (DAMPs) [Boller and Felix, 2009, Zipfel, 2009]. The perception of an invading pathogen leads to pattern-triggered immunity (PTI), which usually stops the infection. Some pathogens have evolved to
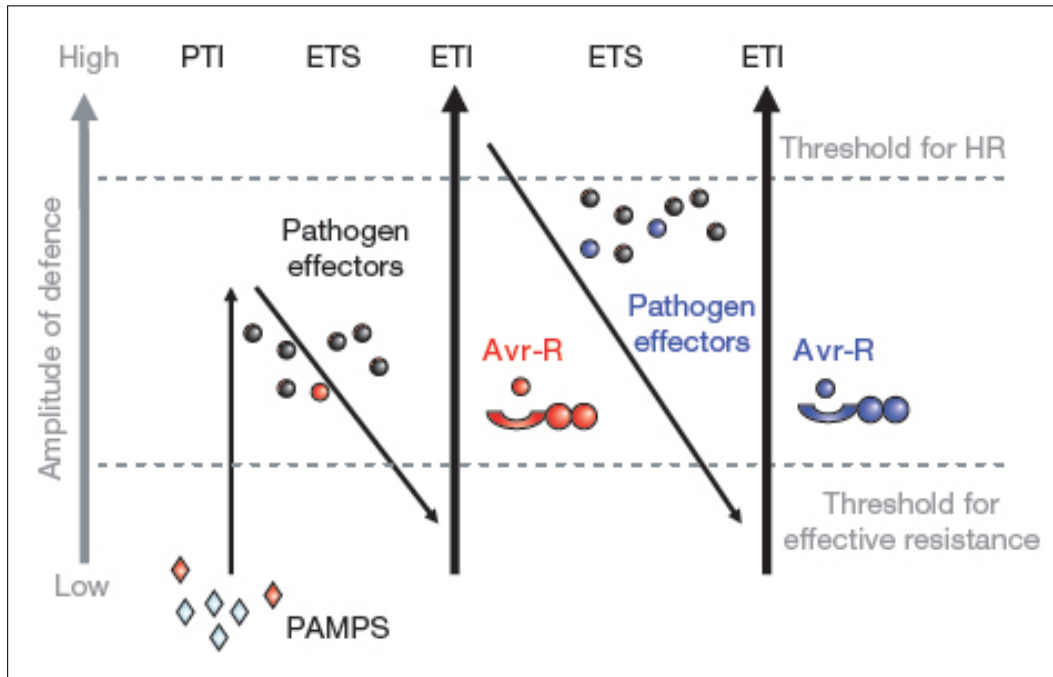
Figure 1.1: A zigzag model of the plant immune system. In phase 1, plants detect PAMPs (red diamonds) via PRRs to trigger PAMP-triggered immunity (PTI). In phase 2, successful pathogens deliver effectors that suppress PTI and lead to effector-triggered susceptibility (ETS). In phase 3, one effector (shown in red) is detected by NB-LRR proteins, leading to effector-triggered immunity (ETI). In phase 4, pathogens are selected that have lost the red effector and perhaps gained new effectors (shown in blue), which can help suppress ETI. Selection favours plant NB-LRR proteins that can recognise the new effectors, once again resulting in ETI. [Jones and Dangl, 2006]

suppress PTI by secreting effector or avirulence (Avr) proteins into the plant cell, resulting in effector-triggered susceptibility (ETS) [Bardoel et al., 2011, de Jonge et al., 2010]. The remaining weak immune response, termed basal defence, is not sufficient to stop the pathogen [Abramovitch et al., 2006]. In response to Avr proteins, plants have developed resistance (R) proteins that recognise effectors, leading to effector-triggered immunity (ETI) or R-gene mediated resistance [Jones and Dangl, 2006]. This is usually accompanied by

the hypersensitive response (HR), a form of programmed cell death (PCD) at the infection site. Pathogens avoid ETI by losing or diversifying effectors or by acquiring new effectors that suppress ETI. Plants and pathogens are constantly evolving, the plants to successfully defend themselves against invading pathogens and the pathogens to suppress the plant defence response [Chisholm et al., 2006].

1.1.1 PAMP-triggered immunity (PTI)

PTI is triggered upon recognition of PAMPs, which are structurally conserved microbial molecules. They are indispensable for the pathogen and usually not present in the host. PAMPs include flagellin [Gómez-Gómez and Boller, 2002], elongation factor Tu (EF-Tu) [Kunze et al., 2004], lipopolysaccharide (LPS) [Zeidler et al., 2004] and also fungal components such as chitin [Baureithel et al., 1994, Day et al., 2001] or ergosterol [Granado et al., 1995]. PAMPs can be recognised by PRRs in plants. Two different types of PRRs have been identified, receptor-like kinases (RLKs) and receptor-like proteins (RLPs) [Shiu and Bleecker, 2001, Shiu and Bleecker, 2003]. PTI is associated with mitogen-acitvated protein kinase (MAPK) signalling, induction of pathogen-responsive genes, production of reactive oxygen species (ROS) and callose deposition at the site of infection to reinforce the cell wall [Nürnberger et al., 2004, Boller and Felix, 2009].

Flagellin, the main component of the bacterial flagellum, is one of the most extensively studied PAMPs [Gómez-Gómez and Boller, 2002]. It induces defence responses in *Drosophila* [Lemaitre et al., 1997], in mammals [McDer-

mott et al., 2000], as well as in plants. In plants, a conserved 22 amino acid region (flg22) in the N-terminal domain of flagellin is recognised by the Flagellin-sensing 2 (FLS2) receptor [Felix et al., 1999], a leucine-rich-repeat receptor-like kinase (LRR-RLK) [Gómez-Gómez and Boller, 2002]. Orthologs of FLS2 have been found in all higher plants studied so far [Boller and Felix, 2009] and functional homologues have been identified in *Arabidopsis* [Gómez-Gómez and Boller, 2000], tomato [Robatzek et al., 2007], *Nicotiana benthamia* [Hann and Rathjen, 2007] and rice [Takai et al., 2008]. In *Arabidopsis*, flg22 induces callose formation, accumulation of PR1 protein and inhibits seedling growth [Gómez-Gómez et al., 1999]. flg22 treatment of *Arabidopsis* induces production of microRNA miRNA393, which targets the auxin receptors TIR1, AFB2, and AFB3, and thereby represses auxin signalling [Robert-Seilaniantz et al., 2011b, Navarro et al., 2006]. Overexpressing miRNA393 makes plants more resistant to biotrophic pathogens, and more susceptible to necrotrophic pathogens.

Arabidopsis and other *Brassicaceae* are able to recognise elongation factor Tu (EF-Tu), one of the most conserved proteins in bacteria [Kunze et al., 2004]. EF-Tu is the most abundant protein in the bacterial cytoplasm and is essential for protein translation. The peptides elf18 and elf26, corresponding to the acetylated N-terminus of EF-Tu, have been shown to trigger PAMP responses in *Arabidopsis* [Kunze et al., 2004].

EF-Tu is recognised by the receptor EFR, which is a LRR-RLK with a similar structure to FLS2 [Shiu and Bleecker, 2003]. Lipopolysaccharide (LPS) is the main component of the outer membrane of gram-negative bacteria. Both

the lipid A part of LPS [Zeidler et al., 2004] as well as oligorhamnans, which are part of the variable O-chain in LPS [Bedini et al., 2005], can trigger defence responses in *Arabidopsis*, suggesting there might be more than one perception system for LPS in Arabidopsis.

The earliest responses to PRR activation include ion fluxes [Boller, 1995] and an increase in ROS [Chinchilla et al., 2007]. ROS can act directly against invading pathogens or indirectly by causing cell wall crosslinking. Furthermore they might act as a stress signal and induce defence responses [Apel and Hirt, 2004]. Another early response to PAMP signals is the activation of MAPK cascades [Nürnberger et al., 2004], leading to the activation of WRKY-type transcription factors [Asai et al., 2002]. MPK3, MPK4, and MPK6 are the best characterised MAPKs in *Arabidopsis* and in addition to their role in plant defence they also play a crucial role in plant growth and development [Pitzschke et al., 2009]. MPK3/MPK6 are necessary for defence signalling downstream of the flagellin receptor FLS2 and are involved in defence responses against bacterial and fungal pathogens [Asai et al., 2002]. Both MPK3 and MPK6 are also required for camalexin biosynthesis in response to *Botrytis cinerea* infection [Ren et al., 2008b] as well as for ABA-mediated stomatal closure in response to drought [Wang et al., 2007], while MPK3 is required for stomatal closure in response to pathogen infection [Gudesblat et al., 2007]. MPK4 negatively regulates SA and H_2O_2 production [Brodersen et al., 2006]. Changes in protein phosphorylation have also been observed as a response to PRR activation [Peck et al., 2001, de la Fuente van Bentem and Hirt, 2007]. Two different studies have identified a number of membrane proteins that are phosphorylated

in response to flg22 and both found that Respiratory burst oxidase homologue D (RbohD) is among the phosphorylated proteins [Benschop et al., 2007, Nühse et al., 2007]. In *Arabidopsis*, treatment with flg22 and elf26 caused the induction of almost 1000 genes and the downregulation of approximately 200 genes within 30 minutes [Zipfel et al., 2004, Zipfel et al., 2006]. In *Arabidopsis* leaves treated with flg22, callose deposition was observed approximately 16 hours after treatment [Gómez-Gómez et al., 1999].

1.1.2   Effector-triggered susceptibility (ETS)

Gram-negative phytopathogenic bacteria use a type III secretion system to deliver effector proteins into the plant cytosol to suppress plant defence responses, which leads to pathogen growth [Bonas and Lahaye, 2002, Collmer et al., 2002, Mudgett, 2005]. Several effectors have been studied in detail. The effector protein AvrRpt2 is a cysteine protease which cleaves Arabidopsis RIN4 [Axtell et al., 2003], and HopPtoD2 is a tyrosine phosphatase that suppresses HR and *PR* gene expression [Bretz et al., 2003, Espinosa et al., 2003]. *Xanthomonas* XopD is constitutively active cysteine protease that hydrolyses plant-specific SUMO-protein conjugates [Hotson et al., 2003]. HopPtoM, AvrE and DspA/E are suppressors of SA-mediated cell wall-based defences [DebRoy et al., 2004], whereas AvrPto suppresses SA-independent cell wall-based defences [Hauck et al., 2003]. AvrPtoB has been shown to act as a general PCD suppressor [Abramovitch et al., 2003]. AvrPto and AvrPtoB interact with the FLS2 receptor and its co-receptor BAK1. AvrPtoB is a ubiquitin ligase, which catalyses the polyubiquitinylation and subsequent proteasome-

dependent degradation of FLS2, a process which is enhanced during flg22 binding to FLS2 [Mersmann et al., 2008]. AvrPto interacts with BAK1 and prevents its binding to FLS2 [Shan et al., 2008]. HopAI1, which is a phospho-threonine lyase, represses signalling by MAPK cascades by dephosphorylating the threonine residue at which the MAPKs MPK3 and MPK6 are activated by their upstream MAPKKs [Zhang et al., 2007].

RPM1-interacting protein 4 (RIN4), a plasma membrane anchored protein, plays a key role in plant immunity and is targeted by multiple TTEs, including AvrB, AvrRpm1, AvrRpt2, AvrPto, AvrPtoB, and HopF2 [Fig. 1.2] [Deslandes and Rivas, 2012]. AvrB and AvrRpm1 both promote phosphory-lation of RIN4, which is detected by the R protein RPM1 and triggers ETI [Mackey et al., 2002, Chung et al., 2011]. AvrB also targets RIN4-interacting
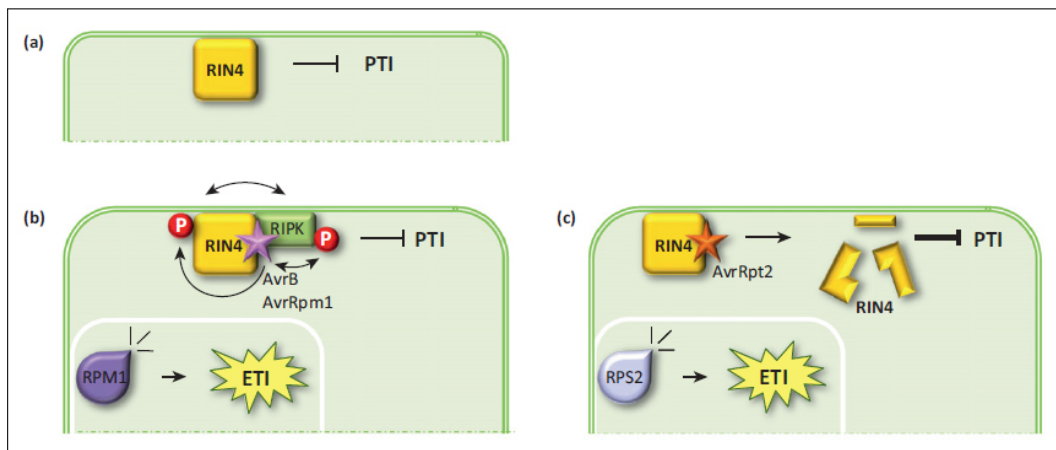
Figure 1.2: Multiple effectors target RIN4. (a) In the absence of pathogen infection, RIN4 negatively regulates PTI. (b) AvrB and AvrRpm1 phospho-rylate RIN4, which is recognised by the R protein RPM1 and triggers ETI. (c) AvrRpt2 cleaves RIN4, the resulting non-membrane anchored fragments of RIN4 are more effective in suppressing PTI than uncleaved RIN4. Adapted from [Deslandes and Rivas, 2012]

receptor-like protein kinase (RIPK), which might enhance phosphorylation of RIN4 by RIPK [Liu et al., 2011]. AvrRpt2 is a cysteine protease, which once inside in the plant cell is self processed into an active protein after interaction with cyclophilin ROC1 [Coaker et al., 2005]. AvrRpt2 cleaves RIN4 and produces non-membrane anchored fragments of RIN4, which are more effective in suppressing PTI than uncleaved RIN4 [Kim et al., 2005, Afzal et al., 2011]. RIN4 proteolysis is detected by the R protein RPS2 [Axtell and Staskawicz, 2003]. AvrRps4 is recognised by the R protein RPS4 [Gassmann et al., 1999], which associates with Enhanced disease susceptibility1 (EDS1) and intercepts AvrRps4 [Heidrich et al., 2011]. EDS1 also interacts with other proteins, such as the R proteins RPS6 [Kim et al., 2009] and SNC1 (Suppressor of npr1-1 constitutive 1) [Zhu et al., 2010], and SRFR1, which negatively regulates ETI [Kwon et al., 2009]. AvrRps4 disrupts EDS1 association with RPS4, RPS6, SNC1, and SRFR1 [Bhattacharjee et al., 2011].

1.1.3 Effector-triggered immunity (ETI)

Plants possess Resistance (R) proteins, which recognise TTEs and then trigger defence responses. There are five different classes of R proteins [Fig. 1.3]. The largest class of R genes encode 'nucleotide-binding site plus leucine-rich repeat' (NB-LRR) proteins, which function exclusively as R genes [Dangl and Jones, 2001]. These proteins have a variable number of C-terminal leucine-rich repeats (LRRs), which play a role in protein-protein interaction, peptide-ligand binding and protein-carbohydrate interaction [Kajava, 1998]. They also possess a conserved nucleotide-binding (NB) site, which in other proteins has

been shown to be critical for ATP or GTP binding [Saraste et al., 1990]. NB-LRR proteins can be divided into two classes based on the structure of their N-terminal domain. The first contain a domain with homology to the intracellular signalling domains of the *Drosophila* Toll and mammalian interleukin-1 receptors (TIR-NB-LRR), whereas the second have putative coiled-coil domains (CC-NB-LRR) [Dangl and Jones, 2001]. Unlike NB-LRR proteins, the other four classes of R genes also have functions in cellular and developmental processes unrelated to health [Dangl and Jones, 2001].

Direct interactions between effector proteins and R proteins have been shown in some cases but in many cases a direct interaction could not be ob-
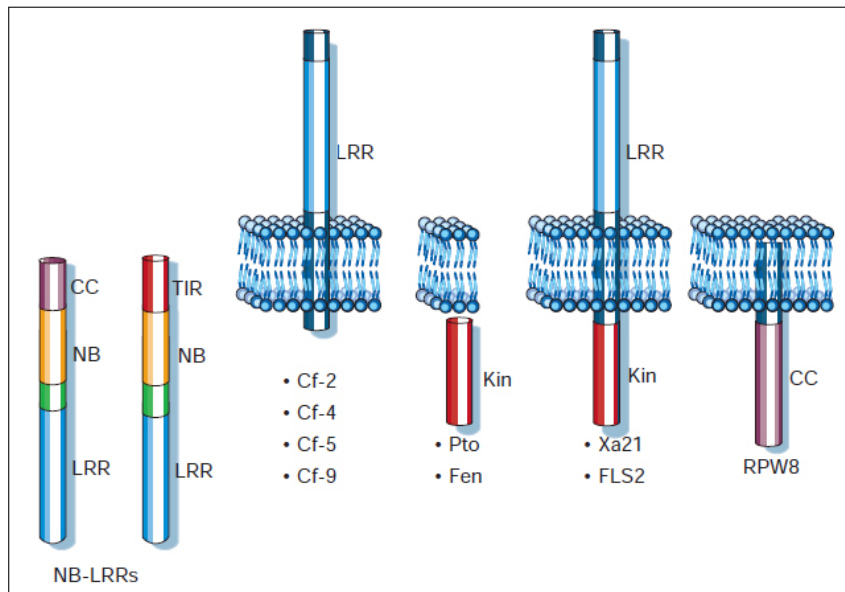
Figure 1.3: Location and structure of the five main classes of plant disease resistance proteins. NB-LRR proteins are presumably cytoplasmic. Cf and Xa21 possess transmembrane domains and extracellular LRRs. The Pto protein is a cytoplasmic Ser/Thr kinase but is possibly membrane-associated through its N-terminal myristoylation site. RPW8 contains a putative signal anchor at the N-terminus. [Dangl and Jones, 2001]

served. To explain this phenomenon, the guard hypothesis was developed, which states that R proteins may recognise effector proteins indirectly by monitoring or 'guarding' effector targets [Dangl and Jones, 2001]. RPM1 interacting protein 4 (RIN4) is a very good example for an effector target that is guarded by NB-LRR R proteins. The *Arabidopsis Resistance to P. syringae pv tomato 1* (*RPM1*) gene confers resistance against *P. syringae* strains expressing either of two TTEs, AvrRpm1 or AvrB. RPM1 guards RIN4, a negative regulator of basal defence responses. AvrRpm1 and AvrB phosphorylate RIN4, which may enhance RIN4 activity as a negative regulator of plant defence and thereby facilitating pathogen growth [Mackey et al., 2002]. RIN4 is also the target for another *P. syringae* effector, AvrRpt2. AvrRpt2 assembles with RIN4 and the R protein Resistant to P. syringae 2 (RPS2), which confers resistance against strains expressing AvrRpt2 [Axtell and Staskawicz, 2003]. RIN4 is a negative regulator of RPS2 and RPM1, possibly keeping these R proteins in an inactive conformation in the absence of a pathogen [Belkhadir et al., 2004, Day et al., 2005]. RIN4 also interacts with the GPI-anchored protein Non-race specific disease resistance protein 1 (NDR1) and activation of both RPM1 and RPS2 requires NDR1 [Day et al., 2006]. Another example is the *P. syringae* effector AvrPphB, which proteolytically cleaves the *Arabidopsis* protein kinase PBS1. This cleavage is then detected by the NB-LRR R protein Resistant to *P. syringae* 5 (RPS5), which confers resistance against bacterial strains expressing AvrPphB [Shao et al., 2003]. In tomato, the protein kinase Pto is a general component of host defence, possibly in a pathway for response to nonspecific elicitors (PAMPs) of phytopathogenic bacteria. The *P. syringae* effector protein AvrPto targets Pto in order to suppress PTI. This interaction is detected

by the NB-LRR protein Prf, which then activates a defence response [Van der Biezen and Jones, 1998, Dangl and Jones, 2001].

There are also some examples for direct interaction between effector proteins and R proteins. The *Pi-ta* gene in rice confers resistance against strains of the rice blast fungus, *Magnaporthe grisea*, expressing the effector protein Avr-Pita in a gene-for-gene relationship [Jia et al., 2000]. In *Arabidopsis*, physical interaction between RRS-1R, a protein conferring resistance against several strains of *Ralstonia solanacearum*, and PopP2, a TTE targeted to the nucleus, has been shown [Deslandes et al., 2003]. The flax *L* locus alleles encode NB-LRR proteins, which interact in yeast with the corresponding AvrL proteins. Both *L* and AvrL are under diversifying selection [Dodds et al., 2006].

EDS1 and NDR1 are essential for the function of different *R* genes, which could suggest the existence of at least two different *R* gene mediated signalling pathways in *Arabidopsis* [Aarts et al., 1998]. The *ndr1* and *eds1* mutants were found in screens for loss of resistance to the bacterium *P. syringae* or to the oomycete *Peronospora parasitica* [Century et al., 1995, Parker et al., 1996]. *eds1* suppresses TIR-NB-LRR *R* genes and *ndr1* suppresses a subset of CC-NB-LRR *R* genes [McDowell et al., 2000]. There are also several CC-NB-LRR proteins that function independently of both EDS1 and NDR1 [McDowell et al., 2000, Aarts et al., 1998].

EDS1 is involved in basal resistance towards biotrophic and hemi-biotrophic pathogens and is also required for TIR-NB-LRR R protein triggered resistance [Wiermer et al., 2005]. EDS1 is located downstream of TIR-NB-LRR activation and upstream of defence gene induction, SA accumulation and host

cell death [Feys et al., 2001, Wirthmueller et al., 2007]. There are two EDS1 isoforms in Col-0 which function redundantly in pathogen defence [Zhu et al., 2011]. EDS1 forms complexes in the cytoplasm and in the nucleus with its defence co-regulators Phytoalexin deficient 4 (PAD4) and Senescence associated gene 101 (SAG101) [Feys et al., 2001, Feys et al., 2005], with ternary complexes of EDS1-PAD4-SAG101 having been observed in some studies [Zhu et al., 2011]. PAD4 and SAG101 seem to be involved in regulating the subcellular localisation of EDS1 [Zhu et al., 2011]. Furthermore, EDS1 and PAD4 have been shown to transduce ROS-derived signals in biotic and abiotic stress signalling [Rustérucci et al., 2001, Mateo et al., 2004]. Flavin-dependent monooxygenase 1 (FMO1) and NUDT7, a member of a cytosolic Nudix hydrolase family, have been identified as components of an EDS1-dependent but SA-independent branch of plant defence and function as a positive and a negative regulator, respectively [Bartsch et al., 2006]. After infection with avirulent bacteria, nuclear enrichment of EDS1 can be observed, which precedes EDS1-dependent transcriptional reprogramming [García et al., 2010]. Among the upregulated genes are *ICS1*, *PBS3*, *CBP60g*, and *PR1*, which are involved in SA biosynthesis and signalling [Wildermuth et al., 2001, Okrent et al., 2009, Wang et al., 2009, Laird et al., 2004], as well as *FMO1*, a positive regulator of a SA-independent defence pathway [Bartsch et al., 2006, Mishina and Zeier, 2006]. Several genes are repressed by EDS1, including *DND1*, a negative regulator of plant innate immunity [Clough et al., 2000], and *ERECTA*, a receptor-like kinase required for resistance to the bacteria *Ralstonia solanacearum* and necrotrophic fungi [Godiard et al., 2003, Llorente et al., 2005]. Both EDS1 and PAD4, but not their physical interaction, are required for HR cell death, while SAG101 is not re-

quired to trigger HR [Rietz et al., 2011]. Basal resistance to virulent pathogens requires direct interaction between EDS1 and PAD4, but can be mostly accomplished without SAG101 [Rietz et al., 2011]. The EDS1-PAD4 complex is also required for full induction of SAR [Rietz et al., 2011]. It has been suggested that after infection with an avirulent pathogen, cell death is triggered by low levels of EDS and by disassociated PAD4, while EDS1 and PAD4 in a complex are involved in the transcriptional reprogramming of cells surrounding the death foci [Rietz et al., 2011]. The latter then leads to activation of SA-mediated defences and establishment of SAR. EDS1 and SAG101, possibly as a complex, are involved in reinforcing resistance at the edges of the local HR.

Changes in ion fluxes, including calcium influx, occur within minutes of R gene activation [Jabs et al., 1997]. Subsequently, ROS (including H_2O_2 and/or $O_2^{\bullet-}$) are produced and MAPK and other protein kinase pathways are activated [Ligterink et al., 1997, Grant et al., 2000]. The ROS are most likely involved in both pathogen elimination and subsequent disease signalling. In addition to ROS, nitric oxide (NO) has been shown to accumulate through a so far unknown biosynthetic pathway [Delledonne et al., 1998, Delledonne et al., 2001]. Within 15 minutes, new transcripts ($\sim$1% of total messenger RNA) encoding signalling molecules such as protein kinases and transcription factors can be observed [Durrant et al., 2000]. Furthermore, biosynthesis of SA, induction of ethylene biosynthesis, cell-wall strengthening, lignification, production of various antimicrobial compounds, and a form of rapid cell death called the hypersensitive response (HR) occur [Scheel, 1998, Lam et al., 2001].

These events not only lead to local resistance to infection but can also lead to systemic acquired resistance (SAR) [Delaney, 1997].

1.1.4 The hypersensitive response

The hypersensitive response (HR), a form of programmed cell death, occurs in response to R gene activation, and it is thought to restrict growth of biotrophic pathogens by cutting off nutrient supply [Lamb and Dixon, 1997]. During the early stages of HR a rapid increase in cytosolic Ca^{2+} is observed [Chandra et al., 1997, Jabs et al., 1997], followed by accumulation of NO [Delledonne et al., 1998, Durner et al., 1998] and ROS [Grant et al., 2000], the latter being generated mainly by NADPH oxidases [Torres et al., 2002]. A balanced production of NO and ROS is needed to trigger HR cell death, and high levels of ROS and NO alone are not sufficient to increase cell death [Delledonne et al., 1998, Delledonne et al., 2001, Zaninotto et al., 2006]. HR is regulated by the interaction of NO with H_2O_2, which is produced from $O_2^{\bullet-}$ by superoxide dismutase (SOD) [Delledonne et al., 2001]. $ONOO^-$, which is formed through interaction of NO with $O_2^{\bullet-}$, is not capable of regulating cell death. During HR, SOD activity increases to avoid a loss of NO by reaction with $O_2^{\bullet-}$ and to faciliate HR through interaction of NO with H_2O_2. S-nitrosylation has also been shown to be involved in H_2O_2-regulated cell death [Wang et al., 2013]. The NADPH oxidase AtRbohD is S-nitrosylated when SNO levels are high, which abolishes its ability to synthesise ROS [Yun et al., 2011]. The Peroxiredoxin PrxII E, which can detoxify $ONOO^-$, is regulated by S-nitrosylation during the defence response [Romero-Puertas et al., 2007].

Several proteins involved in regulating HR have been identified using mutant screens. The *Arabidopsis lesions simulating disease 1* (*lsd1*) mutant exhibits runaway cell death when challenged with avirulent bacteria and is very sensitive to superoxide ($O_2^{\bullet-}$). LSD1 upregulates Cu/Zn SOD, which detoxifies superoxide produced during pathogen infection [Jabs et al., 1996, Kliebenstein et al., 1999] . LSD1 also suppresses cell death by interacting with LSD-One-Like 1 (LOL1) and the AtbZIP10 transcription factor, both of which have been shown to positively regulate HR [Epple et al., 2003, Kaminaka et al., 2006]. The *Arabidopsis* protein RAR1 is required for full HR cell death and full disease resistance mediated by RPM1 and other R proteins [Tornero et al., 2002, Hubert et al., 2003]. RAR1 proteins bind to HSP90, a molecular chaperone [Takahashi et al., 2003], and it has been suggested that RAR1 and HSP90 stabilise R proteins [Hubert et al., 2003]. SGT1b, a conserved adaptor protein, interacts with RAR1, HSP90, and LRR domains of R proteins [Austin et al., 2002, Takahashi et al., 2003, Bieri et al., 2004], and also with SKP1 and CUL1, which are subunits of an E3 ubiquitin ligase complex [Azevedo et al., 2002]. E3 enzymes ubiquitylate proteins, which are then degraded by the 26S proteasome [Vierstra, 2003]. It has been speculated that HR might be initiated by ubiquitination of cell death suppressors and their subsequent degradation by the proteasome [Mur et al., 2008a]. The *defence, no death 1* (*dnd1*) mutant does not show HR, but is still resistant to avirulent bacterial pathogens and shows increased resistance to a broad range of virulent fungal, bacterial, and viral pathogens, suggesting that there are two different pathways involved in cell death and defence gene induction [Yu et al., 1998]. The gene mutated in *dnd1*, *AtCNGC2*, encodes a cyclic nucleotide-gated ion channel, which allows

transport of ions such as Ca^{2+} [Leng et al., 1999, Clough et al., 2000]. A double mutant in the *Arabidopsis* NADPH oxidases *AtRbohD* and *AtRbohF* shows decreased accumulation of ROS and less cell death, but is not impaired in disease resistance [Torres et al., 2002], which also points towards the existence of two different pathways for cell death and defence gene induction.

1.2 Disease signalling

Various plant hormones are involved in disease signalling after pathogen recognition [Bari and Jones, 2009, Katagiri and Tsuda, 2010, Pieterse et al., 2009]. The main defence hormones are salicylic acid (SA) and jasmonic acid (JA) [Vlot et al., 2009, Browse, 2009, Reymond and Farmer, 1998] but other hormones, such as ethylene (ET) [van Loon et al., 2006a], abscisic acid (ABA) [Ton et al., 2009], gibberellins (GAs) [Navarro et al., 2008], auxins [Kazan and Manners, 2009], cytokinins (CKs) [Walters and McRoberts, 2006], brassinosteroids [Nakashita et al., 2003], and nitric oxide (NO) [Moreau et al., 2010] also play an important role in plant defence. There is extensive cross talk between the different plant hormones and they can interact antagonistically or synergistically [Jaillais and Chory, 2010, Mundy et al., 2006, Koornneef and Pieterse, 2008].

The SA pathway regulates defence against biotrophic and hemibiotrophic pathogens, while JA and ET regulate immune responses against necrotrophic pathogens and insect herbivores [Glazebrook, 2005]. The SA and JA pathways are antagonistic [Spoel et al., 2003], and cross talk between these signalling

pathways is controlled by a complex regulatory network [Glazebrook et al., 2003, Sato et al., 2010, Tsuda et al., 2009]. This SA-JA cross talk enables the plant to prioritise one pathway over the other depending on the type of pathogen it is challenged with and to allocate resources accordingly [Bostock, 2005, Kunkel and Brooks, 2002, Verhage et al., 2010]. Plants infected by SA-inducing biotrophic pathogens suppress JA-dependent defences [Felton and Korth, 2000, Spoel et al., 2007], while activation of the JA pathway can suppress SA signalling [Brooks et al., 2005, Uppalapati et al., 2007]. This obviously comes at a cost; induction of the SA pathway by infection with avirulent hemibiotrophic bacteria makes *Arabidopsis* more susceptible to a subsequent infection by a necrotrophic fungus in neighbouring tissues, but not in systemic tissues [Spoel et al., 2007]. In addition to its role in plant defence, SA-JA cross talk also plays a role in adaptive responses to abiotic stresses [Clarke et al., 2009, Ballaré, 2011, Ritsema et al., 2010].

1.2.1 Jasmonic acid mediated signalling

JA is a key signalling molecule which plays a role in growth, development and environmental responses [Browse, 2009], as well as in defence responses against most necrotrophic microorganisms and herbivorous insects [Ballaré, 2011]. Recognition of herbivore- and damage-associated molecular patterns (HAMPs, DAMPs) [Felton and Tumlinson, 2008] triggers JA biosynthesis [Howe and Jander, 2008]. JA is synthesised via the oxylipin biosynthesis pathway [Gfeller et al., 2010, Wasternack, 2007], and then metabolised to methyl jasmonate (MeJa) [Seo et al., 2001] or conjugated to isoleucine by JA conjugate syn-

thase (JAR1) to form the bioactive jasmonoyl-isoleucine (JA-Ile) [Staswick and Tiryaki, 2004, Fonseca et al., 2009b].

There are two branches of the JA signalling pathway; the MYC branch and the ERF branch. The MYC branch is activated by herbivorous insects and wounding [Lorenzo et al., 2004], while the ERF branch is associated
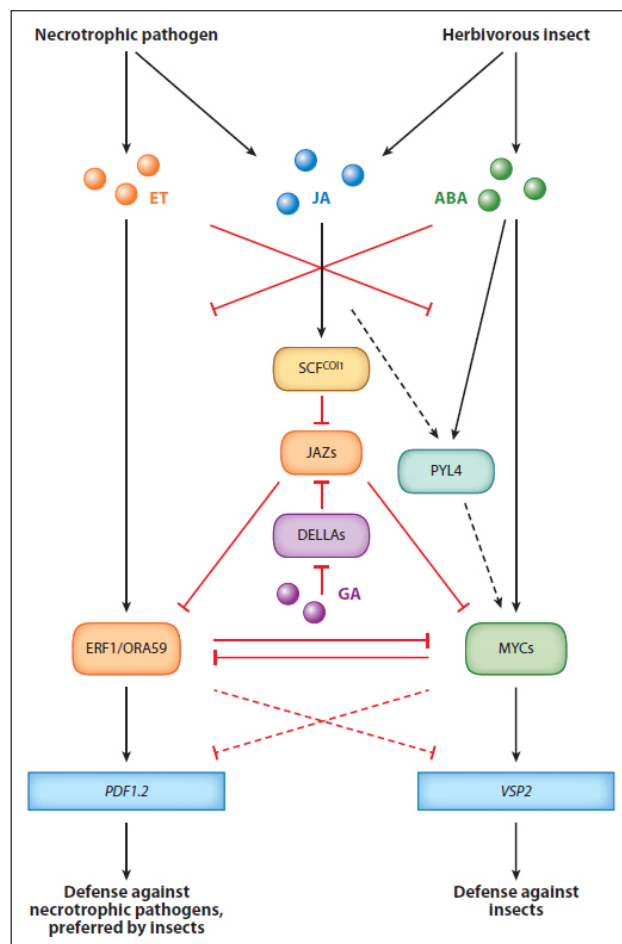
Figure 1.4: Necrotrophic pathogens induce the JA/ET pathways, while insect herbivores induce the JA/ABA pathways. These two branches of the JA pathway are mutually antagonistic. Solid lines, established interactions; dashed lines, hypothesised interactions; arrows, positive effects; red inhibition lines, negative effects. Abbreviations: ABA, ascisic acid; ET, ethylene; GA, gibberelin; JA, jasmonic acid. [Pieterse et al., 2012]

with resistance to necrotrophic pathogens [Fig. 1.4] [Berrocal-Lobo et al., 2002]. These two branches are mutually antagonistic [Pieterse et al., 2012]. The MYC branch is regulated by MYC-type transcription factors and includes the JA-responsive gene *Vegetative storage protein 2* (*VSP2*) [Dombrecht et al., 2007, Lorenzo et al., 2004], while the ERF branch is regulated by Apetala2/Ethylene response factor (AP2/ERF) transcription factors and includes the JA-responsive gene *Plant defensin 1.2* (*PDF1.2*) [McGrath et al., 2005]. In uninduced cells, Jasmonate ZIM (JAZ) proteins together with corepressors such as Topless (TPL) and Histone deacetylase 6 (HDA6) repress JA-responsive genes [Chung et al., 2009, Pieterse et al., 2012]. JA-Ile recognition by the jasmonate receptor Coronatine insensitive (COI1) [Yan et al., 2009] leads to degration of the JAZ repressors and subsequent activation of JA-responsive genes [Fonseca et al., 2009a, Howe, 2010]

JAZ proteins repress JA signalling by binding to positive transcriptional regulators, such as the basic helix-loop-helix transcription factors MYC2, 3, and 4 [Fernández-Calvo et al., 2011, Niu et al., 2011] or the AP2/ERF transcription factors ERF1 and Octadecanoid responsive *Arabidopsis* 59 (ORA59) [Fig. 1.5] [Pré et al., 2008]. JAZ proteins contain two highly conserved regions, a C-terminal Jas domain and a central ZIM domain [Chini et al., 2007, Thines et al., 2007]. The Jas domain is involved in protein-protein interactions with transcription factors and COI1. The adaptor protein Novel interactor of JAZ (NINJA) interacts with the ZIM domain of most JAZ proteins, and through its ERF-associated amphiphilic repression (EAR) motif it recruits the co-repressor TPL [Pauwels et al., 2010, Kazan, 2006]. The F-box protein COI1 binds to
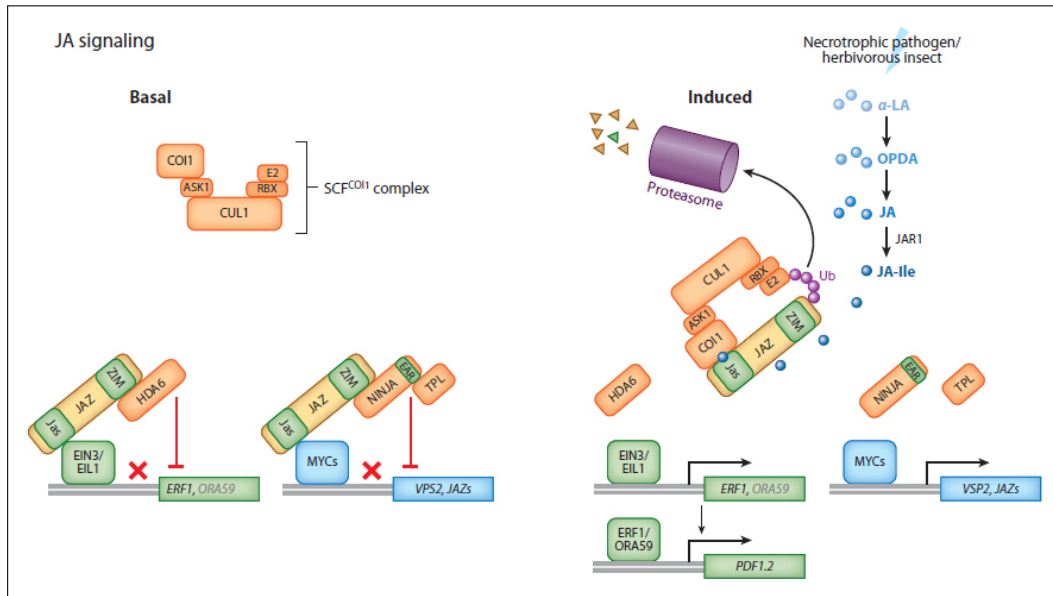
Figure 1.5: JA signalling in uninduced (basal) and induced cells. Solid arrows represent established activities, red inhibition lines and crosses show repression of transcription. *ORA59* is a hypothesised target of EIN3/EIL1. Abbreviations: Ub, ubiquitin. [Pieterse et al., 2012]

other proteins, including SKP1 and Cullin (CUL), to form the multiprotein E3 ligase complex SCF^{COI1} [Xie et al., 1998, Xu et al., 2002]. A complex of COI1 and JAZ has been identified as the true JA-Ile receptor; an open pocket in COI1 recognises JA-Ile, but a loop region in JAZ is needed to trap the hormone [Sheard et al., 2010]. Binding of JA-Ile to COI1 leads to polyubiquitinylation and subsequent degradation of JAZ repressor proteins by the 26S proteasome [Moon et al., 2004, Pauwels and Goossens, 2011], which stops the JAZ-mediated repression of the JA signalling pathway and leads to the expression of JA-responsive genes [Fig. 1.5] [Memelink, 2009].

After local activation of JA signalling, JA responses are induced systemically in distal tissues to protect the plant from future attacks [Howe and Jander, 2008, Koo et al., 2009]. Beneficial soil microorganisms can also induce

a primed state in distal aboveground plant tissue, which makes plants more resistant against a broad spectrum of pathogens and herbivorous insects [Van Wees et al., 2008, Van der Ent et al., 2009, Pineda et al., 2010]. This so-called induced systemic resistance (ISR) results in faster and stronger JA-dependent defence in the event of a pathogen or insect attack.

1.2.2   Salicylic acid mediated signalling

Salylic acid (SA) is crucial in defence against biotrophic or hemibiotrophic pathogens [Glazebrook, 2005], and a complex genetic regulatory network is involved in SA-mediated signalling [Vlot et al., 2009]. Upon pathogen challenge, components of the shikimate pathway are strongly upregulated. Chorismate derived from this pathway is then synthesised to SA by Isochorismate synthase 1 (ICS1) [Truman et al., 2006].

Signalling downstream of most CC-NBS-LRR R proteins is regulated by Non-race specific disease resistance protein 1 (NDR1) [Century et al., 1997, Aarts et al., 1998] [Fig. 1.6]. *ndr1* mutants show suppressed ETI and PTI, whereas overexpression of *NDR1* significantly enhances bacterial disease resistance [Coppinger et al., 2004]. NDR1 is a glycophosphatidyl-inositol-anchored plasma membrane protein [Coppinger et al., 2004], which mediates the activation of disease resistance pathways via a physical interaction with RPM1-interacting protein 4 (RIN4) [Shapiro and Zhang, 2001, Day et al., 2006]. Disease resistance mediated by the CC-NB-LRR R proteins RPS2, RPM1, and RPS5 requires NDR1, but only RPS1 and RPM1 also require RIN4 [Day et al., 2006]. RPS2, RPM1, and also NDR have been shown to interact with RIN4

in planta [Day et al., 2006].

The lipase-like protein Enhanced disease susceptibility 1 (EDS1) and its sequence-related partner Phytoalexin deficient 4 (PAD4) are involved in PTI as well as in ETI initiated by TIR-NB-LRR R genes [Wiermer et al., 2005] [Fig. 1.6]. SA application leads to defence gene induction in *eds1* and
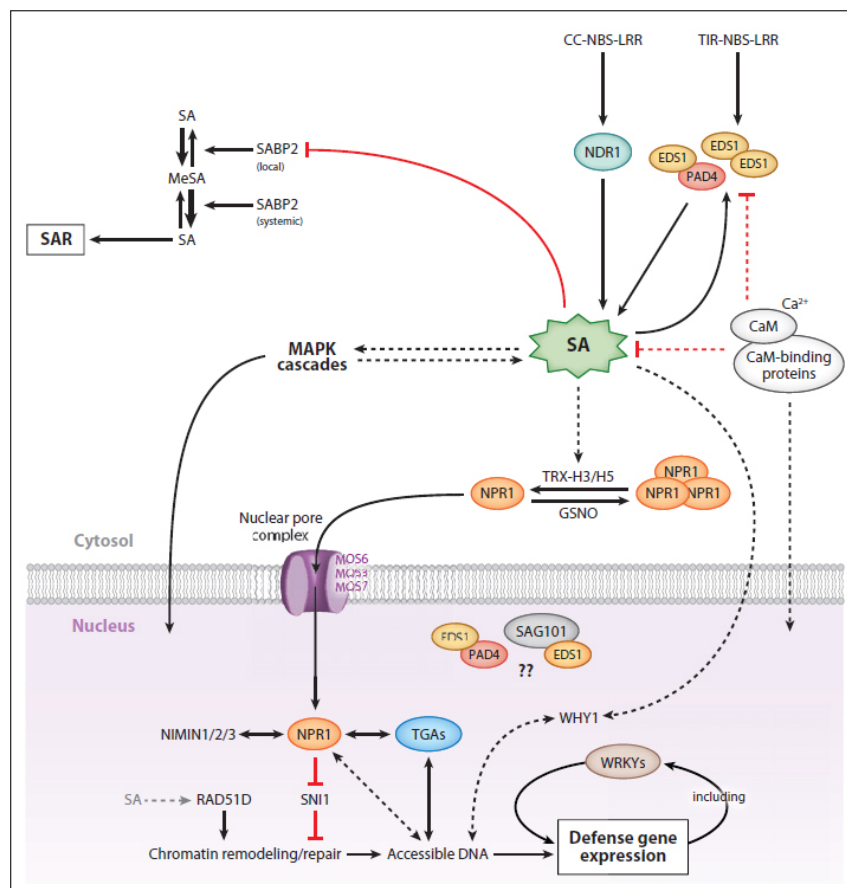
Figure 1.6: Schematic overview of SA signalling. Arrows indicate activation of enzymes, accumulation of compounds, induction of gene transcription, or movement of NPR1 from the cytosol to the nucleus. Double-headed arrows indicate physical protein-protein or protein-DNA interactions. Red lines indicate repression of enzymatic activities or accumulation of compounds. Solid lines indicate established interactions, whereas dashed lines indicate hypothesised or less well characterised interactions. [Vlot et al., 2009]

pad4 plants, indicating that EDS1 and PAD4 lie upstream of SA [Zhou et al., 1998, Falk et al., 1999]. Both EDS1 and PAD4 are required for SA accumulation and for processing ROS-derived signals around infection sites [Feys et al., 2001, Rustérucci et al., 2001]. SA also contributes to the expression of EDS1 and PAD4 through a positive feedback loop which seems to play an important role in defence amplification [Xiao et al., 2003, Chandra-Shekara et al., 2004]. EDS1 and RPS4 together act as a receptor for AvrRps4; nuclear accumulation of EDS1 and RPS4 is necessary for disease resistance, whereas nucleo-cytoplasmatic coordination of EDS1 and RPS4 is required for HR cell death and transcriptional defence amplification [Heidrich et al., 2011].

Non-expressor of PR genes 1 (NPR1), also known as NIM1 or SAI1, plays a central role in SA signalling and is a transcriptional coactivator of many defence-related genes, so-called *PR* (*Pathogenesis-related*) genes [Dong, 2004, Durrant and Dong, 2004, Pieterse and Van Loon, 2004, Moore et al., 2011], which include *PR1*, a commonly used marker gene for SA-dependent gene expression, as well as WRKY transcription factors which can positively or negative regulate SA signalling [Rushton et al., 2010, Wang et al., 2006]. Some *PR* genes encode proteins with antimicrobial properties [van Loon et al., 2006b]. In uninduced cells NPR1 is predominantly present as oligomers in the cytosol; SA-induced redox changes lead to monomerisation of NPR1 and translocation of NPR1 monomers into the nucleus, where they interact with TGA transcription factors and enhance their binding to SA-responsive genes [Mou et al., 2003, Tada et al., 2008]. NPR1 does not contain a DNA binding domain [Cao et al., 1997] but has two putative protein binding domains, an

ankyrin repeat domain and a BTP/POZ (Broad-Complex, Tramtrack, and Bric-A-Brac/Poxvirus, Zinc Finger) domain [Cao et al., 1997,Ryals et al., 1997, Aravind and Koonin, 1999]. The ankyrin repeats mediate interactions with TGA factors, and their mutations abolishes NPR1-TGA complex formation, *PR1* gene expression, and SAR [Rochon et al., 2006]. NPR1 has a functional nuclear localisation signal (NLS) in the C-terminus and mutations in this NLS abolish nuclear accumulation [Zhang et al., 2010].

In unchallenged cells, NPR1 is mostly present in the cytosol as an oligomer held together by disulphide bridges, and oligomerisation of NPR1 is facilitated by S-nitrosylation [Tada et al., 2008]. Small amounts of NPR1 monomers can translocate to the nucleus but these are ubiquitinylated and subsequently degraded by the proteasome to prevent unnecessary defence gene activation [Spoel et al., 2009]. Cytosolic NPR1 plays an important role in the SA-mediated repression of genes involved in JA biosynthesis and signalling, such as LOX2 (Lipoxygenase 2), which encodes a key enzyme in the octadecanoid pathway leading to JA biosynthesis, as well as VSP and PDF1.2 [Spoel et al., 2003]. Nuclear localisation of NPR1 is not required for SA-mediated repression of JA-responsive genes [Spoel et al., 2003].

Translocation of NPR1 monomers into the nucleus is a crucial step in SA signalling, and nuclear localisation of NPR1 is required for activation of *PR* gene expression [Kinkema et al., 2000]. After pathogen perception, SA-induced changes in the cellular redox state lead to reduction of two cysteine residues (Cys82 and Cys216) by Thioredoxin-h5 (Trx-h5) and Trx-h3 [Mou et al., 2003, Tada et al., 2008]. However, the reduction of disulphide bridges

alone is not sufficient to break apart the monomer. NPR1 has recently been shown to bind SA directly through Cys522 and Cys529 via the transition metal copper. SA binding alters the conformation of NPR1 and abolishes interaction between the autoinhibitory N-terminal BTB/POZ domain and the C-terminal transactivation domain of NPR1 [Wu et al., 2012]. NPR1 monomers are then translocated into the nucleus through nuclear pore proteins Modifier of *snc1* MOS 3, 6 and 7 [Cheng et al., 2009, Monaghan et al., 2010], where they interact with TGA transcription factors and activate SA-responsive genes such as *PR1* [Després et al., 2000, Fan and Dong, 2002].

Re-initiation of transcription is necessary to ensure a high rate of *PR* gene expression, so NPR1 is phosphorylated in the nucleus, subsequently ubiquitinylated by CUL3 (Cullin3) E3 ubiquitin ligase, which has a high affinity for phosporylated NPR1, and finally degraded by the proteasome, clearing NPR1 from the promoter so the transcription cycle can be re-iniated [Spoel et al., 2009, Spoel et al., 2010]. SA triggers non-overlapping oxidative and reductive phases, which influence NPR1 oligomer and monomer formation, and lead to re-oligomerisation of NPR1 [Mou et al., 2003, Tada et al., 2008, Spoel et al., 2009]. During the reductive phases, NPR1 activates its target genes [Mou et al., 2003, Tada et al., 2008, Spoel et al., 2009]. Re-oligomerisation of NPR1, which is facilitated by S-nitrosylation of Cys156 of NPR1, is necessary to avoid depletion of NPR1. Plants expressing only a constitutively monomeric form of NPR1 show enhanced disease resistance but are unable to mount an SA-dependent SAR response due to rapid degradation of NPR1 in the nucleus [Mou et al., 2003, Tada et al., 2008].

Nuclear NPR1 is also required for the regulation of salicylate tolerance, ICS1 expression and salicylate accumulation [Zhang et al., 2010]. The TGA triple mutant *tga6 tga2 tga5* is more sensitive to SA toxicity [Zhang et al., 2003], so it is possible that NPR1 may regulate SA tolerance through its interaction with TGA transcription factors. *ICS1* expression may be suppressed through the interaction of NPR1 with WRKY transcription factors. The *ICS1* promoter is enriched in W-box elements, which are binding sites for WRKY transcription factors. During pathogen infection, NPR1 controls the induction of WRKY18, WRKY38 and WRKY62 [Wang et al., 2005], two of which (WRKY38 and WRKY62) are negative regulators of plants basal resistance [Kim et al., 2008].

There are 10 TGA transcription factors in Arabidopsis [Jakoby et al., 2002], seven of which (TGA1 - TGA7) have been shown to interact with NPR1 [Kesarwani et al., 2007] [Fig. 1.7]. NPR1 only interacts with TGA1 and TGA4 after SA-induction [Després et al., 2003]. The interaction is dependent on SA-induced changes of the redox environment, which results in the reduction of two cysteine residues in TGA1 and TGA4 [Durrant and Dong, 2004]. TGA1 and TGA4 have functional redundancy and are important for basal resistance [Kesarwani et al., 2007]. SA-induced *PR1* expression is positively controlled by TGA2, TGA5, and TGA6, which act redundantly. In untreated cells, TGA2 acts as a transcriptional repressor of *PR* genes and does not interact with NPR1 [Rochon et al., 2006]. After SA-induction, a ternary complex is formed between *PR1* DNA, TGA2, and NPR1, with NPR1 acting as a TGA2 coactivator as well as a derepressor [Rochon et al., 2006, Boyle et al., 2009]. TGA3 is
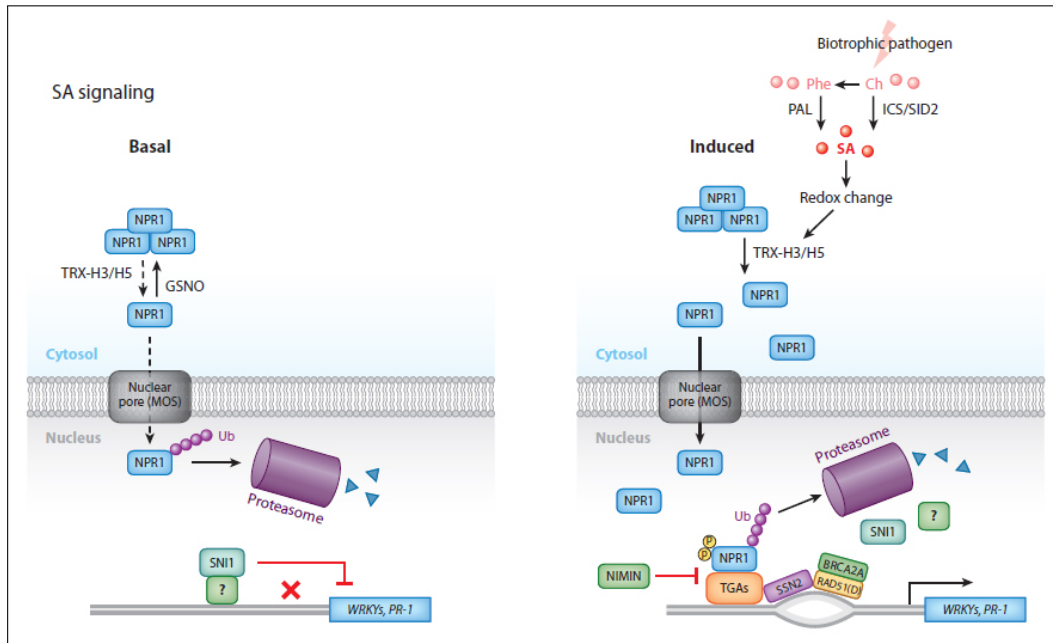
Figure 1.7: SA signalling in uninduced (basal) and induced cells. Solid arrows represent established activities, dashed arrows indicate fairly low activities, red inhibition lines and crosses show repression of transcription. Abbreviations: Ch, chorismate; P, phosphorylated protein; Phe, phenylalanine; Ub, ubiquitin. [Pieterse et al., 2012]

a transcriptional activator for basal and induced *PR* gene expression. The function of TGA7 is currently unclear but it can be speculated that it has a redundant role with TGA3 because of its sequence homology [Kesarwani et al., 2007].

NPR1 also interacts with NIM-interacting1 (NIMIN1), 2, and 3, and Suppressor of *npr1* inducible 1 (SNI1) [Li et al., 1999, Pape et al., 2010, Weigel et al., 2005], all of which are negative regulators of SA-induced *PR* gene expression [Fig. 1.7]. NIMIN genes are not expressed in untreated plants but are transiently induced after SA treatment [Weigel et al., 2001]. NIMIN proteins interact with TGA transcription factors, and possibly play a role in fine-tuning

PR gene induction [Weigel et al., 2005]. SNI1 associates with defence gene pro-moters [Song et al., 2011]. After activation of SA signalling, SNI1 is removed from the promoter, possibly through its interaction with Suppressor of *sni1* 2 (SSN2) and RAS associated with diabetes 51D (RAD51D). A complex of RAD51 (a paralog of RAD51D) and Breast cancer 2A (BRCA2A) is also re-cruited to the *PR1* promoter, and together with SSN2 and RAD51D positively regulates defence gene expression [Durrant et al., 2007, Song et al., 2011, Wang et al., 2010] [Fig. 1.7].

In addition to controlling expression of *PR* genes and genes involved in defence, NPR1 also directly controls the expression of the protein secretory pathway proteins, most of which are localised in the endoplasmatic reticulum (ER) [Wang et al., 2005]. During SAR, high levels of PR proteins are accumu-lated in vacuoles and in the apoplast and a coordinated up-regulation of the protein secretory machinery is needed to ensure proper folding, modification and transport of PR proteins.

Four SA binding proteins have been identified in tobacco, with SABP2 having the highest affinity for SA [Du and Klessig, 1997]. Binding of SA to SABP2 inhibits its MeSA esterase activity, thereby facilitating MeSA accumu-lation in the infected tissue for transport to uninfected tissue [Forouhar et al., 2005, Park et al., 2007]. SA also binds to catalase (CAT) and cytosolic ascor-bate peroxidase (APX), which inhibits the H_2O_2-degrading activity of these two enzymes and leads to increased H_2O_2 levels [Chen et al., 1993, Dempsey et al., 1999]. Another SA binding protein in tobacco is SABP3, a chloroplastic carbonic anhydrase (CA) [Slaymaker et al., 2002]. *Arabidopsis* CA has been

shown to be S-nitrosylated, which suppresses its SA binding and enzymatic acitivity. CA enzymatic activity is required for resistance and S-nitrosylation of CA could be part of a negative feedback loop to modulate the defence response [Wang et al., 2009]. Recently NPR3 and NPR4 were identified as the SA receptors in *Arabidopsis* [Fu et al., 2012]. NPR3 and NPR4 bind SA, and mediate NPR1 degradation through their function as adaptors of the Cullin 3 ubiquitin E3 ligase. Ca^{2+} and calmodulin (CaM) have also been shown to play an important role in regulating SA accumulation and signalling. Binding of Ca^{2+}/CaM to the transcription factor SR1 represses expression of *EDS1* and also suppresses SA accumulation [Du et al., 2009], whereas binding of CaM to PTI-induced CaM-binding protein CPB60g leads to increased SA accumulation and pathogen resistance [Wang et al., 2009].

Genes regulated downstream of SA can be divided into early (within 30 minutes) and late SA-responsive genes. Unlike late SA-responsive genes, expression of early SA-responsive does not require *de novo* protein synthesis and also does not require functional NPR1 [Uquillas et al., 2004]. The best characterised late SA-inducible gene is *PR1*. Four of the TGA factors that interact with NPR1 differentially regulate *PR1* expression in *Arabidopsis* [Kesarwani et al., 2007]. *PR1* is positively regulated in an SA-dependent, but NPR1-independent, manner by the the transcription factor WHY1 [Desveaux et al., 2004]. *PR1* is negatively regulated by Suppressor of NPR1-inducible (SNI1) [Li et al., 1999]. The *PR1* promoter contains both positive and negative cis-regulatory elements, LS5, LS7, and LS10 [Lebel et al., 1998]. In addition to TGAs, SA signalling is also regulated by several members of the WRKY fam-

ily of transcription factors [Eulgem, 2005, Eulgem and Somssich, 2007]. Most of the WRKY factors have opposing effects on SA and JA signalling, which indicates that they are nodes of convergence for SA- and JA-mediated signals in plant defence [Li et al., 2004, Li et al., 2006, Xu et al., 2006, Eulgem and Somssich, 2007].

Mitogen-activated protein kinase (MAPK) cascades play an important role in many signal transduction pathways in plants, as well as in mammals and fungi. In *Arabidopsis*, stress signalling mainly involves AtMPK3, -4, and -6. AtMPK3 and -6 positively regulate SA signalling, while AtMPK4 is a negative regulator [Colcombet and Hirt, 2008]. AtMPK4, which is activated by *Pseudomonas* infection or by flg22 treatment [Fig. 1.8], suppresses SA signalling and activates JA signalling. AtMPK4 might play a role in fine-tuning AtMPK3- and AtMPK6 mediated defence responses associated with PTI [Petersen et al., 2000, Qiu et al., 2008]. MAPK signalling may also play a role upstream of SA or as part of a SA positive feedback loop, because overexpression of the MAPK kinase MKK7 leads to enhanced resistance against biotrophic pathogens, higher *PR1* expression, and the induction of SAR [Zhang et al., 2007a]. The *Enhanced disease resistance 1* (*EDR1*) gene, which encodes a putative MAPKKK, also seems to function upstream of SA because expression of the *nahG* transgene or mutations in *NPR1*, *PAD4* or *EDS1* block *edr1*-mediated resistance [Frye et al., 2001]. EDR1 shows similarity to CTR1, a negative regulator of ethylene responses, and might be part of a MAPK cascade that negatively regulates SA-induced defences.

Multiple feedback loops are involved in SA signalling and they could

play a role in amplifying plant defence responses [Shah, 2003]. While the activation of *R* gene mediated defence signalling induces SA synthesis and downstream defence responses, application of SA also leads to the expression of genes encoding TIR-NB-LRR proteins [Shirano et al., 2002]. *EDS1* expression, which is located downstream of TIR-NB-LRR proteins, is also upregulated
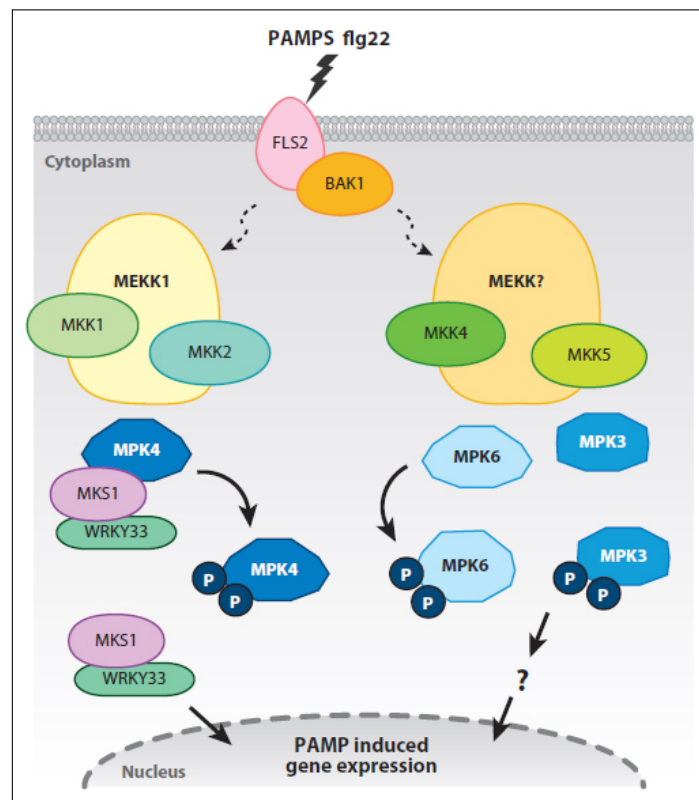
Figure 1.8: MAPK cascades in PAMP-triggered immunity. MEKK1 activates MKK1 and MKK2, which then phosphorylate MPK4. MPK4 forms a complex with its nuclear substrate MKS1 and the transcription WRKY33. Phosphorylation of MPK4 leads to the release of WRKY33. MKK4 and MKK5 activate MPK3 and MPK6, independent of MEKK1 and possibly in a redundant manner. MPK4 acts a negative regulator of PTI, while MPK3 and MPK6 act as positive regulators. Abbreviations: MEKK, MAP kinase kinase kinase; MKK, MAP kinase kinase; MPK, MAP kinase; MKS1, MAP Kinase Substrate 1 [Rodriguez et al., 2010]

by SA [Feys et al., 2001]. SA induces expression of *RPW8*, which confers resistance to the powdery mildew pathogen [Xiao et al., 2003]. EDS5, PAD4 and SID2, which are involved in SA biosynthesis, are also activated by SA [Verberne et al., 2000, Feys et al., 2001, Wildermuth et al., 2001]. SA levels after pathogen infection are higher in *npr1* plants than in wild type plants, indicating that NPR1 is involved in a negative feedback loop which regulates SA accumulation [Delaney, 1997, Shah et al., 1997].

The relationship between SA and ROS is complicated and it has been proposed that SA and H_2O_2 form a self-amplifying feedback loop [Fig. 1.9]. The initial H_2O_2 increase triggered by pathogen infection activates SA synthesis. Increased SA levels together with ROS generated during the second phase of the oxidative burst lead to cell death and defence gene expression. SA also triggers more H_2O_2 production, which then activates the synthesis of more SA and cell death in a self-amplifying loop [Overmyer et al., 2003]. Furthermore, SA interacts with NO, a signalling molecule which is involved in various regulatory processes in mammals and plants [Wendehenne et al., 2004, Grün et al., 2006, Mur et al., 2006a]. In plants, NO and SA seem to function in a positive feedback loop, where NO donors induce SA accumulation and NO defence signalling is dependent on SA [Wendehenne et al., 2004, Grün et al., 2006]. This process is possibly regulated through the S-nitrosylation of proteins, including NPR1 [Loake and Grant, 2007, Tada et al., 2008]. Cross talk between the SA signalling pathway and other hormonal signalling pathways has also been observed [Bostock, 2005, Robert-Seilaniantz et al., 2007]. SA generally antagonises JA signalling; SA signalling is predominantly involved
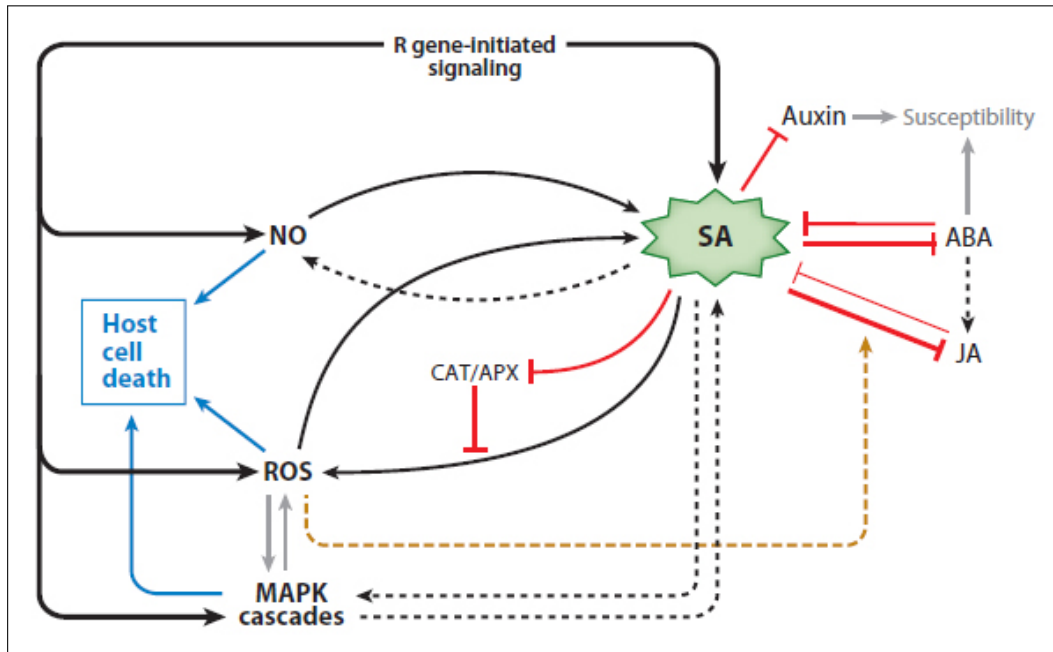
Figure 1.9: SA in relation to other defence signals. Black arrows indicate activation, whereas red lines indicate repression. Solid lines show established interactions, while dashed lines show hypothesised or less well known interactions. [Vlot et al., 2009]

in protection against biotrophic pathogens and viruses, whereas JA signalling mediates resistance against necrotrophic pathogens and insects [Glazebrook, 2005]. SA also suppresses auxin signalling, which reduces susceptibility [Wang et al., 2007]. There is also bidirectional cross talk between SA and absiscic acid (ABA) [Fig. 1.9].

After activation of SA-dependent defence responses at the site of infection, a mobile signal spreads through the plant, leading to a similar SA-dependent response in distal tissues, which leads to a long-lasting broad-spectrum resistance against subsequent pathogen infection, termed systemic acquired resistance (SAR) [Vlot et al., 2009]. Methyl salicylate (MeSA) was thought to be active phloem signal for induction of SAR [Park et al., 2007]

but this was later disproven [Attaran et al., 2009]. JA has also been shown to be involved in SAR [Truman et al., 2006].

It has been shown that the expression profiles corresponding to PTI, ETI, basal defence and SAR are qualitively very similar and that the main differences are of a quantitative or temporal nature, suggesting that different pathogens trigger a common and highly interconnected signalling network [Katagiri, 2004]. Such a network is represented by the WRKY transcription factors, which play a crucial role in disease resistance by regulating and fine-tuning defence responses. They can act as positive or negative regulators, auto-regulate or cross-regulate other WRKY factors and there is a high level of redundancy [Eulgem and Somssich, 2007]. Expresson levels of many WRKY genes change after pathogen infection [Dong et al., 2003], and eight WRKYs (*AtWRKY18*, *38*, *53*, *54*, *58*, *59*, *66*, and *70*) have been shown to be direct targets of NPR1 [Wang et al., 2006]. Among these, *WRKY70* plays a central role, it positively regulates SA-dependent defences, while repressing JA signalling [Li et al., 2004, Li et al., 2006]. Furthermore it is also required for both basal defence and full *R* gene (*RPP4*)-mediated defence against the oomycete *Hyaloperonospora parasitica* [Knoth et al., 2007].

1.2.3 SA-JA cross talk

Interactions between the SA and JA pathways are generally antagonistic but neutral or synergistic interactions can also take place [Mur et al., 2006b, Schenk et al., 2000, van Wees et al., 2000]. Low concentrations of SA and JA have a synergistic effect on *PDF1.2* and *PR-1*, while higher concentrations have

antagonistic effects [Mur et al., 2006b]. A threshold model has been proposed, where a certain threshold of one hormone versus the others is needed to cause a resistance trade-off [Spoel and Dong, 2008], and temporal segregation abolishes this antagonism. The relative concentration of each hormone [Mur et al., 2006b], as well as the timing and sequence of signal initiation is important [Koornneef et al., 2008, Leon-Reyes et al., 2010].
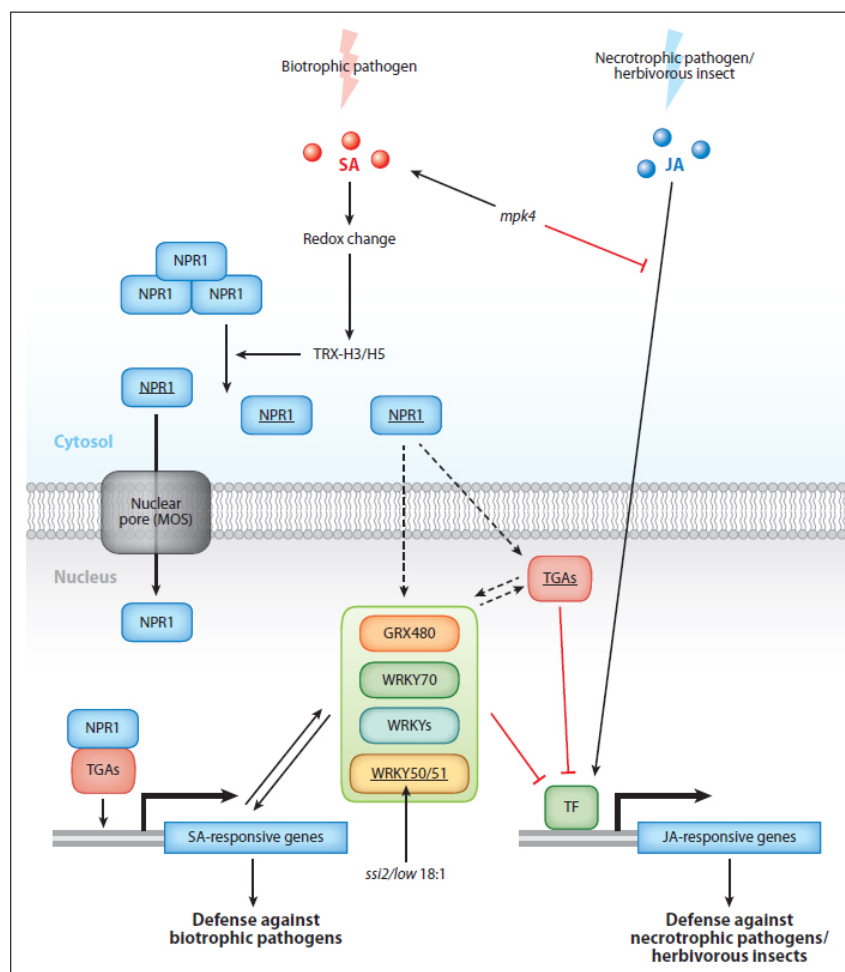
Figure 1.10: SA and JA crosstalk. Underlined, components that have been shown to be essential for SA-JA cross talk; green frame, components for which the expression is SA-responsive; solid lines, established interactions; dashed lines, hypothesised interactions. [Pieterse et al., 2012]

SA-JA cross talk is regulated by many proteins, such as redox regulators, NPR1, TGA and WRKY transcription factors, MAPKs, and SSI2 [Pieterse et al., 2012] [Fig. 1.10]. SA increases the total amount of and ratio between reduced and oxidised glutathione, while JA decreases the glutathione pool [Spoel and Loake, 2011]. SA-induced increases in glutathione levels coincide with repression of the JA pathway, showing that SA-triggered changes in redox status are important for suppression of JA-responsive genes [Koornneef et al., 2008]. Glutaredoxins (GRXs) play an important role in redox regulation of proteins [Foyer and Noctor, 2011, Spoel and Loake, 2011] and GRX480 [Ndamukong et al., 2007] as well as several TGA-interacting GRXs are involved in suppression of the JA pathway [Zander et al., 2012]. NPR1 is a transcriptional coactivator of SA-responsive genes [Wang et al., 2006] and *npr1* mutants are impaired in SA-mediated suppression of the JA pathway [Leon-Reyes et al., 2009, Spoel et al., 2003]. TGA transcription factors regulate the SA-induced expression of *PR* genes [Zhang et al., 2003] and are essential for SA-JA cross talk [Leon-Reyes et al., 2010, Ndamukong et al., 2007, Zander et al., 2010]. After induction of the SA pathway TGAs negatively regulate JA signalling by acting together with MYC2, but in the absence of SA some TGAs can positively regulate JA- and ET-responsive gene expression [Zander et al., 2010]. Many WRKY transcription factors are upregulated by SA and play an important role in SA-dependent defence responses [Rushton et al., 2010]. WRKY50 and WRKY51 are involved in SA-induced suppression of JA signalling [Gao et al., 2011]. WRKY70 is a node of convergence between the SA and JA pathways [Li et al., 2006, Li et al., 2004], it is induced in a SA- and partly NPR1-dependent manner, and it is repressed by JA [Li

et al., 2004]. WRKY70 positively regulates SA signalling, and negatively regulates JA signalling [Li et al., 2004, Ren et al., 2008a]. WRKY62 is a negative regulator of the JA pathway and is SA- and NPR1-inducible [Mao et al., 2007]. Other WRKY transcription factors, such as WRKY8 [Chen et al., 2010], WRKY11 and WRKY17 [Journot-Catalino et al., 2006], WRKY18, WRKY40, and WRKY60 [Xu et al., 2006], and WRKY41 [Higashi et al., 2008], have also been shown to be involved in SA-JA crosstalk. *Arabidopsis* MAP kinase 4 (MPK4), which targets EDS1 and PAD4 [Brodersen et al., 2006], is a negative regulator of SA signalling and a positive regulator of JA signalling [Petersen et al., 2000]. Suppressor of SA insensitivity 2 (SSI2) is a negative regulator of the SA pathway, and a positive regulator of the JA pathway [Kachroo et al., 2003]. SSI2 desaturates stearic acid to oleic acid and is involved in NPR1-independent defence signalling [Kachroo et al., 2001].

Several plant hormones also modulate SA-JA cross talk [Bari and Jones, 2009, Robert-Seilaniantz et al., 2011a]. Ethylene (ET) plays a crucial role in modulating plant immunity [Broekaert et al., 2006, van Loon et al., 2006a, von Dahl and Baldwin, 2007] and there is extensive cross talk between the ET and SA/JA signalling pathways [Glazebrook et al., 2003, Sato et al., 2010]. In *Arabidopsis*, ET potentiates SA/NPR1-induced *PR1* expression [De Vos et al., 2006, Leon-Reyes et al., 2009] and in tobacco it is required for SAR induction [Verberne et al., 2003]. The ET-responsive transcription factors EIN3 and EIL1 repress PAMP-responsive genes, which leads to a decrease of SA accumulation [Chen et al., 2009]. In combination with JA, ET acts synergistically on the ERF branch of the JA pathway, and antagonistically on the MYC

branch [Anderson et al., 2004, Lorenzo et al., 2003, Pré et al., 2008]. Simultaneous induction of the JA and ET pathway makes plants insensitive to future SA-mediated repression of JA signalling [Leon-Reyes et al., 2010], possibly to ensure that the defence response against necrotrophs is not suppressed in the case of a secondary infection by biotrophic pathogens. Abscisic acid (ABA) is involved in development and adaptation to abiotic stresses, but also plays an important role in plant immunity [Asselbergh et al., 2008, Cao et al., 2011, Ton et al., 2009]. It is a negative regulator of SA signalling [de Torres Zabala et al., 2009, Jiang et al., 2010, Yasuda et al., 2008] and together with JA it acts synergistically on the MYC branch of JA signalling and antagonistically on the ERF branch [Abe et al., 2003, Anderson et al., 2004]. JA can positively regulate ABA signalling via the ABA receptor PYL4 [Lackman et al., 2011]. Auxins are crucial for plant development [Benjamins and Scheres, 2008] and can supress SA accumulation and signalling [Robert-Seilaniantz et al., 2011b]. SA, on the other hand, is also capable of suppressing auxin-related genes and auxin signalling, and this plays an important role in the SA-dependent defence against biotrophs [Wang et al., 2007]. Gibberelins (GAs) regulate the degradation of growth-repressing DELLA proteins [Sun, 2011]. DELLA proteins positively regulate the JA pathway by binding to the JA signalling repressor JAZ1, thereby reducing its interaction with MYC2, which can then activate JA-responsive genes [Hou et al., 2010]. Degradation of DELLA proteins makes plants more susceptible to necrotrophs and enhances resistance to biotrophs [Navarro et al., 2008]. Cytokinins (CKs) are growth hormones and positively regulate SA signalling [Choi et al., 2011, Robert-Seilaniantz et al., 2011a]. The CK-activated transcription factor ARR2 binds to the SA-

responsive transcription factor TGA3, which then positively regulates *PR1* expression [Choi et al., 2011].

Many pathogens produce phytohormones or phytohormone mimics to exploit SA-JA cross talk to their advantage and suppress the host's defence mechanisms [Costacurta and Vanderleyden, 1995]. *Ralstonia solanacearum* produces ET and auxin-related compounds [Valls et al., 2006], *Streptomyces turgidiscabies* produces CK [Joshi and Loria, 2007], *Botrytis cinerea* produces ABA [Marumo et al., 1982] and CK [Tudzynski and Sharon, 2002], and *Fusarium oxysporum* produces ABA [Dorffling et al., 1984]. Many strains of *Pseudomonas syringae* produce coronatine (COR), which is a molecular mimic of JA-Ile [Nomura et al., 2005]. COR binds to the JA receptor COI1 [Katsir et al., 2008], thereby activating JA signalling, which antagonises SA-dependent defence responses [Brooks et al., 2005] and also inhibits PAMP-triggered stomatal closure [Melotto et al., 2008]. In addition to producing phytohormones themselves, pathogens can also hijack plant signalling pathways to induce hormone production by the host [Grant and Jones, 2009, Robert-Seilaniantz et al., 2011a]; a well known example is *Agrobacterium tumefaciens* [Akiyoshi et al., 1983]. *P. syringae* produces several TTEs that can induce auxin and ABA production [O'Donnell et al., 2003a, Schmelz et al., 2003, Chen et al., 2004]. HopI1 suppresses SA accumulation by localising to the chloroplast, where it interacts with Heat shock protein 70 (HSP70) [Jelenska et al., 2010]. AvrPtoB induces ABA biosynthesis and ABA responses [de Torres-Zabala et al., 2007, de Torres Zabala et al., 2009] and AvrRpt2 alters auxin physiology [Chen et al., 2007], both of which antagonise the SA pathway. Some herbivores such as *Spodoptera*

spp. [Weech et al., 2008, Diezel et al., 2009] and *Bremia tabaci* [Zarate et al., 2007] activate the SA pathway during feeding to repress JA-mediated defence responses, and the necrotrophic fungus *Gibberella fujikuroi* induces production of JA-antagonistic GA [Navarro et al., 2008].

1.3 Nitric oxide

Nitric oxide (NO) is a small, highly diffusible gas and a key signalling molecule in plants [Hong et al., 2008, Lamattina et al., 2003]. It is involved in all kinds of physiological processes, such as germination, root growth, stomatal closing, iron homeostasis, pollen tube growth, and hormonal signalling [Astier et al., 2012], and plays a very important role in plant-pathogen interactions [Mur et al., 2006a, Romero-Puertas et al., 2004, Durner and Klessig, 1999]. NO has a positive effect on germination, leaf extension and root growth, and delays leaf senescence and fruit maturation [Delledonne, 2005]. The nitrosative burst, an increase in reactive nitrogen species (RNS) in response to R gene activation, is involved in defence signal transduction as well as in establishment of the HR [Delledonne et al., 1998, Delledonne et al., 2001, Durner et al., 1998]. NO has been linked with accumulation of SA and JA [Huang et al., 2004, Mur et al., 2008b], induction of defence genes [Grün et al., 2006], MAMP-triggered ABA-mediated stomatal closure [Melotto et al., 2006], and also affects ethylene, ABA and auxin signalling [Lindermayr et al., 2006, Melotto et al., 2006, Correa-Aragunde et al., 2004]. Because NO possesses an unpaired electron, it shows high reactivity with oxygen, superoxide, transition metals and thiols [Fig. 1.11]. Many important regulatory proteins contain thiols at active sites or

points of allosteric regulation. In a process called S-nitrosylation, NO reacts with these thiols to form S-nitrosothiols (SNOs) [Wang et al., 2006]. Metal nitrosylation, where NO forms complexes with metal-containing proteins such as hemoglobin, has also been observed in plants [Besson-Bard et al., 2008, Dordas et al., 2004]. NO reacts with $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$), which is a fairly destructive molecule within biological systems [Stamler et al., 1992].
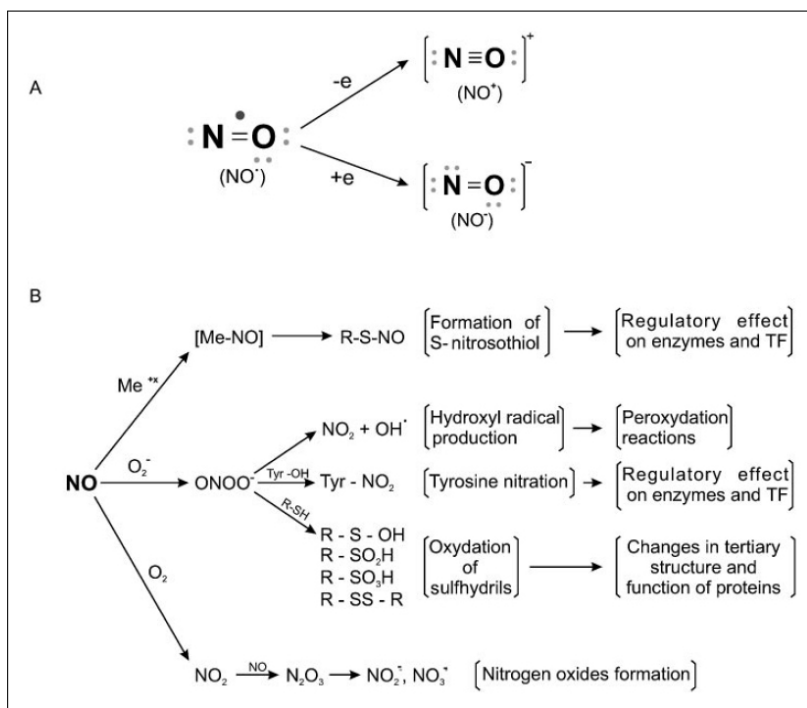
Figure 1.11: NO chemistry. (A) The NO radical ($NO^\bullet$) is oxidised to form the nitrosonium cation (NO^+), or reduced to form the nitroxyl anion (NO^-). (B) NO can react with transition metals (Me^{+x}) such as Fe, Cu, or Zn to form metal-nitrosyl complexes. NO^+ and $NO^\bullet$ can nitrosylate cysteine thiols of proteins (R-S-NO), which plays a role in regulating certain enzymes and transcription factors (TF). NO reacts with $O_2^{\bullet-}$ to form peroxynitrite ($ONOO^-$), which can then lead to the formation of NO_2 and the hydroxyl radical ($OH^\bullet$), as well as tyrosine nitration (Tyr-NO_2) and oxidation of thiols to sulfenic and sulfonic acids. NO reacts with O_2 to form NO_2, which then reacts with NO to form N_2O_3 and then NO_2^-/NO_3^-. [Lamattina et al., 2003]

Protein tyrosine nitration occurs when ONOO$^-$ adds a nitro group to the aromatic ring of tyrosine residues [Radi, 2004, Rubbo and Radi, 2008], which can change protein function or conformation and can also make the protein more susceptible to degradation by the proteasome [Grune et al., 1998, Souza et al., 2000] In plants, increased tyrosine nitration has been observed in response to abiotic and biotic stresses [Corpas et al., 2008, Saito et al., 2006].

1.3.1 NO metabolism

While the route for NO synthesis in animals is well established, it is a lot less clear in plants [Fig. 1.12]. In animals, NO is generated by a family of nitric oxide synthases (NOS) during the conversion of L-arginine to citrulline [Palmer et al., 1993]. Mammals possess three well characterised NOS enzymes, neuronal (n)NOS, endothelial (e)NOS, and inducible (i)NOS [Nathan and Xie, 1994]. Animal NOS catalyse oxidation of arginine to NO and citrulline [Alderton et al., 2001]. A related enzyme, which exhibited NOS activity, has been found in the single-celled green alga *Ostreococcus tauri* but not in any higher plants [Foresi et al., 2010]. In plants, pathogen infection leads to an NO burst, which can be blocked by animal NOS inhibitors [Delledonne et al., 1998]. This finding suggests the presence of an (i)NOS in plants, even though candidate orthologues of animal NOS have not been found so far [Hong et al., 2008]. The *Arabidopsis* gene NOS1, now named Nitric oxide associated 1 (NOA1), was initially reported to show NOS activity [Guo et al., 2003] but this was later disproven [Zemojtel et al., 2006, Crawford et al., 2006]. However, loss of NOA1 function resulted in lowered *in vivo* NO levels after ABA treatment [Guo

et al., 2003] and also compromised the nitrosative burst induced by LPS [Zeidler et al., 2004], suggesting that NOA1 may directly or indirectly regulate NO synthesis. Even though a plant NOS has not been identified yet, NO production from arginine has been observed in higher plant cell extracts and this activity was blocked by animal NOS inhibitors [Corpas et al., 2006]. Furthermore, a loss-of-function mutant *NO overproducer 1 (nox1)* exhibits increased levels of NO, arginine and citrulline [He et al., 2004] as well as increased levels of RNS during *R* gene mediated resistance [Yun et al., 2011], suggesting the existence of a plant NOS. Another enzyme that contributes towards the NO burst during the plant immune response is Nitrate reductase (NR) [Srivastava et al., 2009, Kamoun et al., 1998, Yamamoto-Katou et al., 2006]. Usually this enzyme catalyses the reduction of nitrate to nitrite but it can also catalyse the reduction of nitrite to NO [Rockel et al., 2002, Yamasaki and Sakihama, 2000, Modolo et al., 2005]. In *Arabidopsis*, NR is encoded by two genes, *NIA1* and *NIA2*, with most of the NR activity coming from *NIA2* [Wilkinson and Crawford, 1991]. *nia1 nia2* double mutants show reduced NO levels, both when unchallenged and after challenge with avirulent bacteria [Oliveira et al., 2010]. They also fail to develop HR after pathogen infection and support higher pathogen growth, indicating that NR plays a role in pathogen-induced NO synthesis. However, during aerobic conditions or when nitrite levels are low, NR is not able to synthesise high levels of NO, making it unlikely that NR is the sole source of RNS during the plant immune response [Rockel et al., 2002, Hong et al., 2008]. LPS-triggered NO generation involves a NOS-like enzyme and is NPR1-dependent, but does not require NR [Sun et al., 2012]. NO is still accumulated in *nia1 nia2* in response to LPS and there is no increase in

NR activity in wild type plants after LPS treatment, furthermore supporting the idea that NR is not the only NO source involved in plant immunity. Other possible sources of NO in plants include non-enzymatic reactions [Bethke et al., 2004] and mitochondrial nitrite-dependant NO synthesis [Planchet et al., 2005].

NO is metabolised to nitrate by non-symbotic haemoglobins, such as *Arabidopsis* Hb1, which acts as a NO dioxygenase, using NADPH as an electron donor [Perazzolli et al., 2004]. NO reacts with GSH to form S-nitrosylated glutathione (GSNO), a RNS which acts as a natural reservoir of NO [Liu et al., 2001]. GSNO is involved in transnitrosylation or can release NO, and GSNO
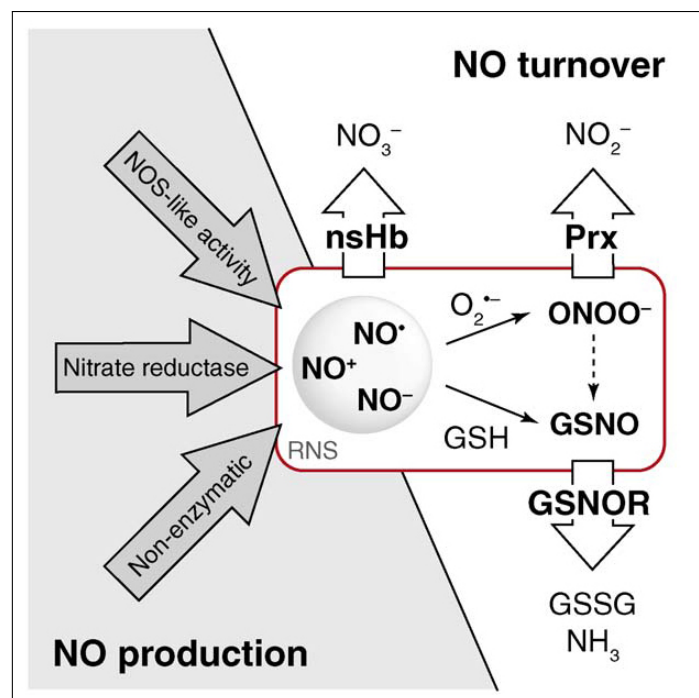
Figure 1.12: NO production and turnover in plants. Three pathways for NO production: A NOS-like activity of an unidentified protein, nitrate reductase, and non-enzymatic conversion of nitrite to NO under acidic conditions. NO is turned over through NO scavenging by non-symbiotic haemoglobins, transformation of NO to GSNO, and reaction of NO with superoxide to form peroxynitrite, which is then detoxified by peroxiredoxins. [Leitner et al., 2009]

levels are controlled by GSNOR, which turns over GSNO by reducing it to oxidised glutathione and NH_3 [Liu et al., 2001]. NO reacts with superoxide ($O_2^{\bullet-}$) to form the highly reactive, oxidising and nitrating, peroxynitrite ($ONOO^-$) in a non-enzymatic reaction [Romero-Puertas et al., 2007]. $O_2^{\bullet-}$ is detoxified by peroxiredoxins, which are targets of and inhibited by S-nitrosylation [Romero-Puertas et al., 2007].

1.3.2 S-nitrosylation

S-nitrosylation, which is the addition of a NO group to a cysteine thiol, has been shown to be involved in the post-translational regulation of many proteins, in plants as well as in animals [Wendehenne et al., 2004, Grün et al., 2006, Hess et al., 2005, Stamler et al., 2001]. SNOs play an important role in human health and elevated or decreased SNO levels have been observed in a variety of diseases [Foster et al., 2003]. While a lot of research was originally focused on animals, it is now becoming clear that S-nitrosylation also plays a crucial role in regulating plant physiological processes, including pathogen defence [Astier et al., 2012, Yu et al., 2012, Besson-Bard et al., 2008, Wang et al., 2006].

SNOs play an important role in regulating SA-dependent gene expression during plant defence [Fig. 1.13] [Malik et al., 2011, Yu et al., 2012]. Upon recognition of avirulent pathogens, SA levels increase and NO is synthesised through an unknown mechanism, possibly by a NOS. NO bioactivity is transduced via S-nitrosylation of reactive protein thiols and GSNOR1 indirectly regulates total SNO levels by turning over GSNO. SABP3 acts as a positive
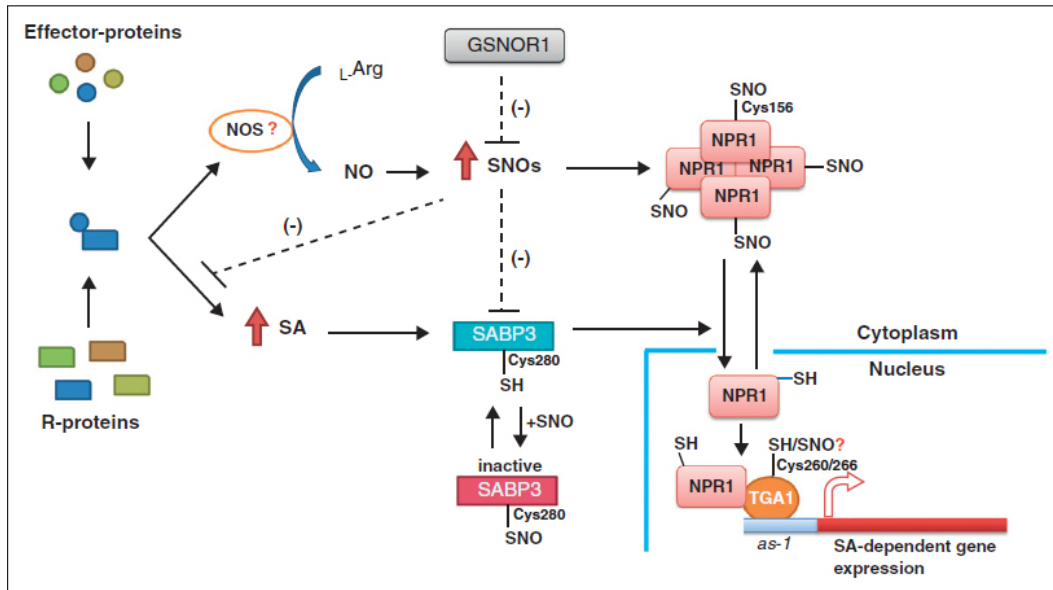
Figure 1.13: Model showing the possible roles of SNOs in regulating SA-dependent gene expression during plant defence. [Yu et al., 2012]

regulator of the defence response. One of the most important S-nitrosylated proteins in SA signalling is NPR1. There is a dynamic equilibrium between NPR1 oligomers and monomers. NPR1 monomers translocate into the nucleus where they bind TGA1 and act as transcriptional co-activators. S-nitrosylation of NPR at Cys156 facilitates oligomerisation, so the equilibrium can be maintained and depletion of NPR1 is avoided. SNO levels increase as a response to pathogen infection and beyond a certain threshold of S-nitrosylation, SA synthesis, SABP3 function and NPR1 monomerisation are blocked, thereby negatively regulating the plant defence response.

Apart from NPR1, several other proteins have been identified as targets for S-nitrosylation. 63 proteins were found to be S-nitrosylated after GSNO treatment of cell cultures and 52 proteins were S-nitrosylated after NO treatment of plants [Lindermayr et al., 2005]. In *Arabidopsis* leaves undergoing HR,

16 proteins were differentially S-nitrosylated [Romero-Puertas et al., 2008]. 11 mitochondrial proteins are also targets for S-nitrosylation [Palmieri et al., 2010]. Another study identified 127 proteins that were S-nitrosylated during challenge with avirulent or virulent bacteria and in GSNO-treated protein extracts [Maldonado-Alconada et al., 2011]. The identified proteins are involved in a wide range of cellular processes, with the largest group of proteins being involed in metabolism (41%), followed by signalling and regulation (14%), cytoskeleton (11%), stresses and pathogen infection (10%), photosynthesis (7%), and redox-related processes (6%). CAT2 was among the S-nitrosylated proteins.

Several S-nitrosylated proteins in *Arabidopsis* have been characterised in detail, including NPR1, TGA1, SABP3, NADPH Oxidase RbohD, Peroxiredoxin II E, GAPDH and Metacaspase MC9. Most of these proteins are to some extent involved in plant immunity. S-nitrosylation of NPR1 faciliates re-oligomerisation of NPR1, so that depletion of cytosolic NPR1 is avoided [Tada et al., 2008]. In unchallenged cells, TGA1 forms an intracellular disulphide bridge which prevents it from interacting with NPR1. Upon SA accumulation, TGA1 becomes reduced, allowing it to bind NPR1 [Després et al., 2003]. Both NPR1 and TGA1 have been shown to be S-nitrosylated after GSNO treatment [Lindermayr et al., 2010]. *Arabidopsis* SABP3 binds SA with high affinity and has also been shown to exhibit Carbonic anhydrase (CA) activity, catalysing the hydration of CO_2 to HCO_3^- [Slaymaker et al., 2002]. After pathogen infection, SABP3 is S-nitrosylated at Cys280, which decreases SA binding and also inhibits its CA activity [Wang et al., 2009]. S-nitrosylation of

SABP3 negatively regulates the plant defence response and it has been speculated that SNO-SABP3 could be part of a negative feedback loop involved in plant immunity. RbohD has been shown to be S-nitrosylated at Cys890 during the plant defence response, which impairs its ability to bind flavin adenine dinucleotide (FAD), thereby blunting its NADPH oxidase activity [Yun et al., 2011]. S-nitrosylation of RbohD during the development of HR results in decreased ROS accumulation, and prevents excessive cell death. This mechanism is evolutionary conserved; human and *Drosophila* NADPH oxidases have also been shown to be S-nitrosylated at this cysteine. Peroxiredoxins reduce H_2O_2 and alkyl hydroperoxides to H_2O and the corresponding alcohol [Dietz, 2003]. Some peroxiredoxins, including PrxII E, also exhibit peroxynitrite reductase activity and detoxify $ONOO^-$. NO-mediated S-nitrosylation of PrxIIE inhibits its peroxidase activity as well as its peroxynitrite reductase activity [Romero-Puertas et al., 2007]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity is inhibited by S-nitrosylation in plants and animals [Lindermayr et al., 2005, Stamler et al., 2001]. H_2O_2 interacts with and inhibits GADPH, suggesting that it might play a role in mediating ROS signalling in plants [Hancock et al., 2005]. In animals, S-nitrosylated GADPH has been shown to be involved in cell death [Hara et al., 2005]. Metacaspase 9 (MC9) is constitutively S-nitrosylated *in vivo*, which leads to it being retained in its inactive, unprossed form [Belenghi et al., 2007]. In its processed, mature form MC9 is not a target for S-nitroslation. MC9 is possibly involved in programmed cell death.

Both NO and SNOs have been shown to be important regulators of H_2O_2-induced leaf cell death in rice [Lin et al., 2012]. Rice NO accumulation mutants

nitric oxide excess 1 (*noe1*) have a mutation in rice catalase *OsCATC*, which corresponds to *Arabidopsis* CAT2. Total catalase levels in *noe1* plants are reduced to about 30% of wild type levels, resulting in increased H_2O_2 levels. The increased H_2O_2 levels lead to an induction of NR (twice wild type level), resulting in higher NO content (2.3 times wild type level). *noe1* plants also show higher SNO content than wild type plants. Overexpression of GSNOR in *noe1* plants decreases cell death, indicating that S-nitrosylation plays a role in H_2O_2-induced cell death. Glyeraldehyde 3-phosphate dehydrogenase (GAPDH) and thioredoxin (TRX), both of which have been shown to be involved in cell death in animals, were only S-nitrosylated in *noe1* plants but not in wild type plants.

1.3.3 Denitrosylation

There are two main enzyme systems that are involved in denitrosylation [Benhar et al., 2009]. The first one is the GSNOR system, which comprises GSH and GSNOR, and the second one is the Thioredoxin (Trx) system, which comprises Trx and Trx reductase (TrxR) [Fig. 1.14]. Transition metal ions and other redox-active species, such as ROS or ascorbate, can also catalyse SNO decomposition [Benhar et al., 2009].

GSNO is an important low-molecular-weight SNO. It is formed either by the reaction between NO (or related species) and GSH, or through GSH-mediated S-nitrosylation of cellular SNOs [Hess et al., 2005]. GSNO is a stable molecule and acts as a reservoir for NO bioactivity [Liu et al., 2001]. GSNO is the major substrate of GSNO reductase (GSNOR), which catalyses the denitro-
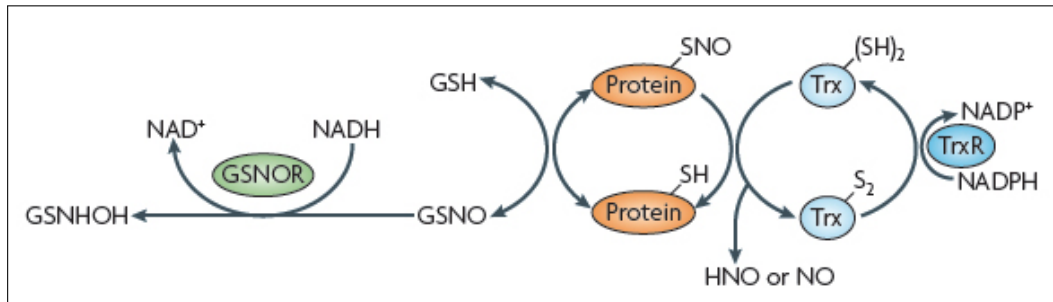
Figure 1.14: Mechanism of protein denitrosylation by Thioredoxin (Trx) and S-nitrosoglutathione reductase (GSNOR). [Benhar et al., 2009]

sylation of GSNO using NADH as an electron donor. The absence of GSNOR results in increased abundance of both GSNO and SNO proteins, although GSNOR does not denitrosylate SNO proteins directly [Liu et al., 2001, Foster et al., 2009]. GSNO and SNO proteins are in equilibrium and GSNOR controls protein SNO levels indirectly through regulating GSNO turnover. GSNO can release NO or transfer the NO group to a target cysteine, in a process called trans-nitrosylation [Singh et al., 1996, Foster et al., 2009]. GSNOR is highly conserved, not just in plants, but also in bacteria and mammals [Liu et al., 2001]. In *Arabidopsis*, GSNOR was initally characterised as Glutathione-dependent formaldehyde dehydrogenase (GS-FDH) but it was later shown that it also exhibits GSNOR activity [Sakamoto et al., 2002]. *GSNOR* expression is down-regulated by wounding in a JA-dependent but ABA-independent manner and it is upregulated by SA [Díaz et al., 2003]. GSNOR also plays an important role in animals, and in humans GSNOR has been shown to be involved in asthma [Staab et al., 2008].

The Trx system, which consists of Trx proteins, TrxR proteins and NADPH, is an important protein disulphide reductase system which is present

in all living organisms. Trx and TrxR proteins play an important role in protecting cells from oxidative stress and are involved in a variety of cellular processes [Lillig and Holmgren, 2007]. Trx can break down GSNO and, unlike GSNOR, it can also directly denitrosylate SNO proteins [Benhar et al., 2009]. Trx play an important role in protecting cells from oxidative damage. In plants, there are a variety of different Trx, which can be found in the cytosol, in chloroplasts and mitochondria, as well as in the nucleus [Vieira Dos Santos and Rey, 2006]. There are 42 *Trx* genes in *Arabidopsis* [Meyer et al., 2005]. Cytosolic Trx-h5, which shows increased expression during infection with avirulent pathogen and in response to oxidative stress, is thought to play a role in the oxidative burst [Laloi et al., 2004]. Many ROS scavening enzymes have been identified as Trx targets [Marchand et al., 2004, Yamazaki et al., 2004, Balmer et al., 2004]. There are also several reductases that are dependent on thioredoxins, such as peroxiredoxins (PRXs), glutathione peroxidases (GPXs), and methionine sulfoxide reductases (MSRs). PRXs detoxify peroxide substrates through oxidation of their active-site cysteine, which is then regenerated by Trx or other reductants [Wood et al., 2003]. GPXs scavenge H_2O_2 and other ROS [Ursini et al., 1995], and it has been shown that several plant GPXs show increased peroxidase activity in the presence of Trx [Herbette et al., 2002, Jung et al., 2002]. MSRs reverse methionine oxidation by catalysing the Trx-dependent reduction of methionine sulfoxide back to methionine [Marchand et al., 2004, Sadanandom et al., 2000, Vieira Dos Santos and Rey, 2006].

1.4 Reactive oxygen species

Reactive oxygen species (ROS) are toxic by-products of aerobic metabolism and can lead to oxidation of lipids, proteins and DNA. Because of photosynthesis, where ROS are produced during electron transport processes, plants have to cope with much higher levels of ROS than animals. In order to protect themselves from oxidative damage, plants developed a variety of protective mechanisms, such as small antioxidant molecules and antioxidant enzymes. These protective mechanisms eventually lead to an elaborate network of ROS producing and scavenging enzymes, which can adjust ROS levels and make it possible for ROS to be used as signalling molecules in a variety of cellular processes, such as such as photosynthesis, cell wall metabolism, defence gene expression, and development [Gechev et al., 2006, Gadjev et al., 2008].

1.4.1 ROS generation and detoxification

ROS are produced during aerobic metabolism by multistep reduction of molecular oxygen (O_2) [Fig.1.15] [Halliwell, 2006, Van Breusegem et al., 2001]. The first step leads to the formation of superoxide ($O_2^{\bullet-}$) or hydroperoxide ($HO_2^{\bullet}$) radicals. $O_2^{\bullet-}$ has a short half life of 2 to 4 μs and in most biological systems it is rapidly converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 is relatively stable with a half life of 1 ms. $O_2^{\bullet-}$ and H_2O_2 can interact in the presence of metal ions to form the highly reactive hydroxyl radical ($HO^{\bullet-}$). In *Arabidopsis*, there are at least 289 genes encoding ROS producing and scavenging enzymes [Gechev et al., 2006].
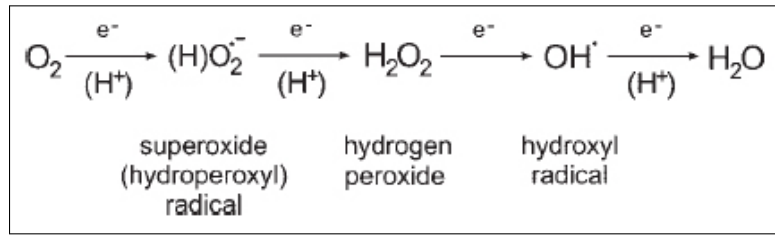
Figure 1.15: Production of ROS by multistep reduction of molecular oxygen. [Gechev et al., 2006]

There are multiple sites and sources of ROS production [Fig. 1.16]. Chloroplasts are the major sites of ROS generation in plants, where singlet oxygen ($^1O_2$) and superoxide anion ($O_2^{\bullet-}$) are produced during photosynthesis. $O_2^{\bullet-}$ is then immediately metabolised to H_2O_2 by SOD [Asada, 2006]. Other important sites of ROS are peroxisomes and glyoxysomes, where ROS are generated during photorespiration and fatty acid oxidation, respectively [del Río et al., 2006]. ROS production in mitochondria is much lower compared to chloroplasts but mitochondrial ROS play an important role as regulators of stress adaption, programmed cell death and other cellular processes [Robson and Vanlerberghe, 2002]. ROS in the apoplast are mainly generated by plasma membrane-bound NADPH oxidases [Sagi et al., 2004]. Apoplastic ROS play an important role in the oxidative burst observed as part of HR [Torres et al., 2002, Torres et al., 2006] and also regulate cell growth, development and cell death [Dat et al., 2000, Gechev and Hille, 2005, Gapper and Dolan, 2006].

The oxidative burst consists mainly of H_2O_2, which is an important signalling molecule in plants [Neill et al., 2002], and the source for it is either NADPH oxidase or apoplastic peroxidase, dependent on plant species, tissue or developmental stage [O'Brien et al., 2012a]. In *Arabidopsis*, both NADPH
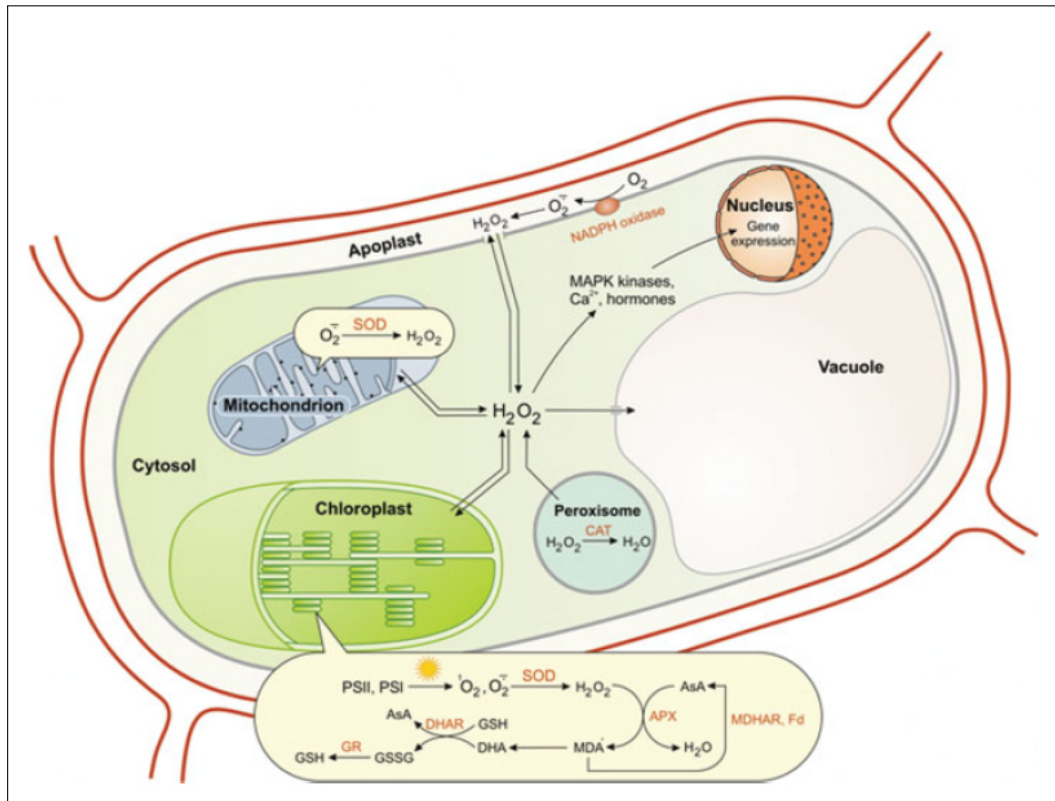
Figure 1.16: Schematic representation of a plant cell depicting major sources of ROS generation and ROS scavenging enzymes. [Gechev et al., 2006]

oxidase as well as peroxidase are involved in ROS production. The majority of ROS is produced by plant NADPH oxidases, called respiratory burst oxidase homologues (Rboh). RbohD and RbohF are required for full oxidative burst in response to infections by avirulent bacteria and oomycete pathogens [Torres et al., 2002, Torres et al., 2005], as well as for defence against virulent bacteria [Chaouch et al., 2012]. However, during PTI peroxidases are the main source of ROS [Daudi et al., 2012, O'Brien et al., 2012b]. The peroxidases AtPrx33 and AtPrx34 are important for maintaining cell wall composition [O'Brien et al., 2012b], and are needed for callose deposition [Daudi et al., 2012]. There are also intracellular sources of ROS, such as mitochondrial ROS

produced during ABA signalling [He et al., 2012]. Photorespiration is another source of ROS, and is tightly regulated by antioxidant enzymes and molecules. *cat2* mutants are more resistant than wild type when grown under long day conditions [Chaouch et al., 2010].

Plants possess an elaborate enzymatic and non-enzymatic antioxidant system which allows them to tightly control ROS levels [Table 1.1]. SODs are the only plant enzymes that are able to scavenge $O_2^{\bullet-}$. H_2O_2 can be catalysed by a variety of different enzymes, either directly by catalases (CAT) or with the help of various reductants by ascorbate peroxidases (APX), peroxiredoxins, glutathione peroxidases (GPX) and guaiacol peroxidases [Dat et al., 2000]. Catalases are only active at relatively high H_2O_2 concentrations, whereas lower H_2O_2 levels are eliminated by APX and other peroxidases [Gechev et al., 2006]. There are also several non-enzymatic antioxidants, such as ascorbate, glutathione, tocopherol and carotenoids [DellaPenna and Pogson, 2006]. It is thought that CAT is responsible for removing high levels of ROS during stress conditions, whereas APX plays a role in fine-tuning ROS levels for signalling [Mittler, 2002]. There is some functional redundancy between the different ROS scavengers. Plants with suppressed APX show induction of SOD, CAT, and GR, whereas plant with supressed CAT exhibit higher levels of APX, GPX, and mitochondrial AOX [Rizhsky et al., 2002, Willekens et al., 1997].

Major plant ROS-associated enzymes and antioxidants

Enzyme/antioxidant (number of genes in *A. thaliana*)	Function	Localisation
Superoxide dismutases (SOD) (8)	Dismutation of $O_2^{\bullet-}$, leads to H_2O_2 formation	cyt, chl, mit, per
Catalases (3)	Detoxifies H_2O_2; no reductor required	mit, per, gly
Ascorbate peroxidases (APX) (9)	Detoxifies H_2O_2 with ascorbate as reductor	cyt, chl, mit, per
Monodehydroascorbate reductases (MDHAR) (5)	Reduces monodehydroascorbate radicals with NAD(P)H as reductor	cyt, chl, mit
Dehydroascorbate reductases (DHAR) (5)	Reduces dehydroascorbate radicals with GSH as reductor	cyt, chl, mit
Glutathione reductases (GR) (2)	Reduces oxidized glutathione with NADPH as reductor	cyt, chl, mit, per
Guaiacol peroxidases (POX) (73)	Detoxifies H_2O_2 with various substrates as reductors; can also produce $O_2^{\bullet-}$, $HO^{\bullet}$, $HOO^{\bullet-}$	cw, cyt, mit, vac
Glutathione peroxidases (GPX) (8)	Detoxifies H_2O_2 and lipid hydroperoxides with GSH as reductor	cyt, chl, mit, er
Glutathione-S-transferases (GST) (53)	Detoxification reactions (Degluthathionylation). Can detoxify lipidhydroperoxides and exhibit DHAR activity.	apo, cyt, chl, mit, nuc
Peroxiredoxins (Prx) (10)	Thiol-containing peroxidases, detoxify H_2O_2	cyt, chl, mit, nuc
Thioredoxins (Trx) (46)	Redox-control of enzymes and transcription factors, electron donor to Prx and GPX	cyt, chl, mit, nuc
Glutaredoxins (Grx) (31)	Deglutathionilation, redox-control of enzymes and transcription factors, electron donor to DHA and Prx	plasmalemma, cyt, chl, mit, er
Ferritins (4)	Binds iron, thus sequestering it in a bioavailable, non toxic form and preventing formation of $HO^{\bullet}$	chl, mit
Alternative oxidases (AOX) (6)	Channels electrons from electron transfer chains of mitochondria and chloroplasts directly to oxygen, thus minimizing $O_2^{\bullet-}$ production under conditions that favour electron transport chain over energization	chl, mit
Ascorbate	Substrate for APX. Detoxifies H_2O_2	apo, cyt, chl, mit, per, vac
Glutathione	Substrate for various peroxidases, glutathione transferases and glutathione reductases. Detoxifies H_2O_2, other hydroperoxides and toxic compounds	apo, cyt, chl, mit, per, vac
α-Tocopherol	Protects membrane lipids from peroxidation, detoxifies lipid peroxides and quenches $^1O_2$	membranes
Carotenoids	Quench $^1O_2$. Photosystem assembly, key components of the light harvesting complex, precursors of ABA	chl, chromoplasts, elaioplasts, amyloplasts
Flavonoids	Can scavenge H_2O_2 and $HO_2^{\bullet}$ directly.	vac

Table 1.1: The abbreviations are: cw, cell wall; apo, apoplast; cyt, cytosol; chl, chloroplasts; mit, mitochondria; er, endoplasmatic reticulum; vac, vacuole; per, peroxisomes; gly, glyoxysomes; nuc, nucleus. Adapted from [Gechev et al., 2006]

1.4.2 The role of ROS in signalling

It appears that plants sense ROS through several different mechanisms: (i) unidentified receptor proteins; (ii) redox-sensitive transcription factors such as NPR1 and HSFs; and (iii) direct inhibition of phosphatases by ROS [Mittler, 2002, Neill et al., 2002, Vranová et al., 2002, Apel and Hirt, 2004]. It appears that there are positive amplification loops involving NADPH oxidases. Low levels of ROS might activate the loops, resulting in enhanced ROS production and amplification of the ROS signals in specific cellular locations [Mittler et al., 2004]. The specificity of the response to altered ROS levels depends on various factors, such as the type of ROS, intensity and duration of the signal, and the site of ROS production. Three different ROS species, ozone, hydrogen peroxide and singlet oxygen, have been shown to induce pathogen resistance in tobacco and *Arabidopsis*. Synthesis of SA and ethylene, local lesions, induction of defence systems (PR proteins, phytoalexins, structural barriers) and ultimately pathogen resistance were observed in response to elevated ROS levels [Sandermann, 2000]. ROS are involved in the regulation of a variety of developmental processes, including root hair growth and elongation, apical dominance, leaf shape, tracheary elements maturation, trichome development, aleurone cell death and senescence [Gechev et al., 2006]. During abiotic stress, ROS scavenging enzymes are induced, leading to a decrease of ROS levels [Mittler et al., 1999]. ROS are also involved in ABA-induced stomatal closure [Pei et al., 2000], as well as in auxin signalling and gravitropism in maize roots [Joo et al., 2001]. ROS and SA signalling also involved in accumulation of camalexin [Chaouch et al., 2010, Daudi et al., 2012, O'Brien

et al., 2012b, Millet et al., 2010], which is one of the major phytoalexins in arabidopsis.

After successful recognition of pathogen a rapid and transient production of ROS is observed, the so-called 'oxidative burst' [Grant and Loake, 2000, Torres, 2010]. The oxidative burst is biphasic, with a first unspecific, transitory phase within minutes after pathogen infection and a second sustained phase hours after infection. The second phase is associated with the induction of plant defences and leads to the hypersensitive response. In addition to ROS production, SA and NO suppress the activity of APX and CAT, leading to a further increase in ROS levels [Klessig et al., 2000, Mittler et al., 1998, Dorey et al., 1998]. ROS signal transduction occurs through MAPK cascades [Kovtun et al., 2000, Samuel et al., 2000]. In *Arabidopsis*, H_2O_2 activates the MAPKs MPK3 and MPK6 via the MAPKKK ANP1 [Kovtun et al., 2000], and also increases expression of nucleotide diphosphate kinase 2 (NDPK2) [Moon et al., 2003]. NDPK2 can interact with and activate the MAPKs. Calmodulin has also been shown to be involved in ROS signalling [Desikan et al., 2001, Harding et al., 1997].

Pathogen infection leads to a dramatic redox change, which is sensed through reversible, oxidative cysteine modifications of regulatory proteins, with the small-molecule redox couples $NAD(P)H/NADP^+$, GSH/GSSG, and ASC/DHASC acting as signalling intermediates [Noctor, 2006, Spoel and Loake, 2011, Yun et al., 2012].There is an electron flow (redox flux) from NAD(P)H to glutathione to ascorbate and the ratio of oxidised versus reduced small-molecule couples changes, which is sensed by reactive cysteines of redox sensor
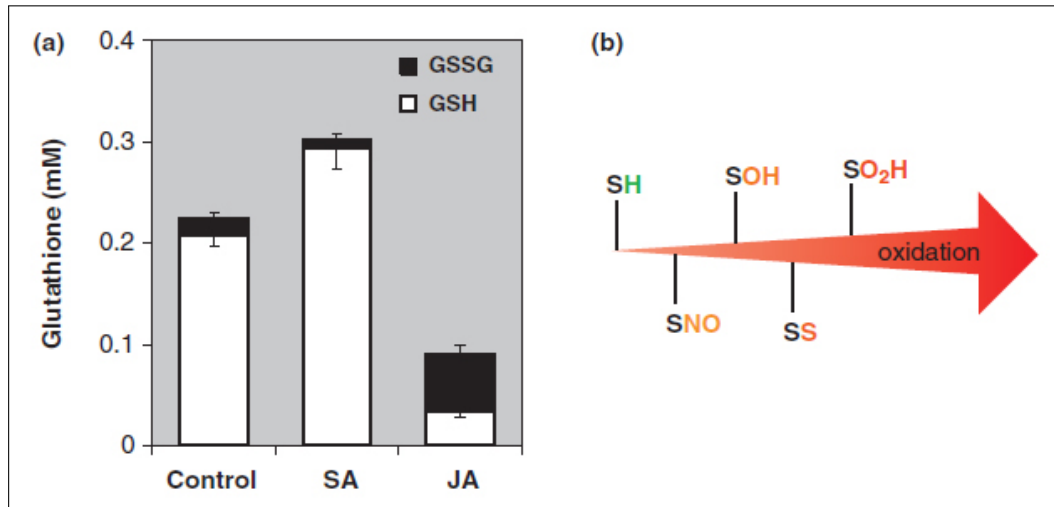
Figure 1.17: Cellular redox status and oxidation status of cysteines (a) The total amount of glutathione as well as the ratio between oxidised (GSSG) and reduced glutathione (GSH) changes in response to defence hormones SA and JA. (b) Different oxidation levels of cysteines: S-nitrosylation (SNO), S-sulphenation)SOH), S-thiolation (SS), and S-sulphination. [Spoel and Loake, 2011]

proteins [Spoel and Loake, 2011]. Particularly glutathione plays an important role in redox signalling during plant defence signalling, as evidenced by the susceptible phenotype of the glutathione-deficient *pad2-1* mutant [Parisy et al., 2007, Schlaeppi et al., 2008, Ball et al., 2004]. Reactive cysteines of regulatory proteins involved in plant immunity are subject to the following reversible post-translational redox modifications, with increasing oxidative levels: S-nitrosylation (SNO, covalent attachment of NO), S-sulphenation (SOH, thiol hydroxylation), S-thiolation (disulphide bridge formation with other protein thiols or with glutathione), S-sulphination (SO_2H, further oxidation of SOH) [Fig. 1.17] [Spoel and Loake, 2011]. The oxidative status can be controlled indirectly by catalases, GSH1 and VTC1 or directly by specific redox enzymes such as isomerases, reductases, nitrosylases, and denitrosylases [Spoel and

Loake, 2011]. Many proteins involved in plant defence are regulated through post-translational redox-based modifications, including proteins involved in immune signalling such as NPR1 [Mou et al., 2003, Spoel et al., 2009, Tada et al., 2008], its interacting transcription factors TGA1 and TGA4 [Després et al., 2003, Lindermayr et al., 2010], and SABP3 [Wang et al., 2009], as well as proteins involved in PCD such as Prx II E [Romero-Puertas et al., 2007] and MC9 [Belenghi et al., 2007].

1.5 Aims

S-nitrosylation plays an important role in plant defence [Feechan et al., 2005]. To gain further insights into how S-nitrosylation is regulated and to possibly uncover new signalling pathways, a suppressor screen of the GSNOR1 loss-of-function mutant *atgsnor1-3* was performed and two suppressor mutants were isolated [Sorhagen, 2010]. The aims of the project were to identify the mutations using map-based cloning and sequencing, to confirm through complementation that the mutations were correctly identified, and to characterise the suppressor mutants to determine what aspects of the *atgsnor1-3* phenotype are suppressed. This includes, but is not limited to, characterising the suppressor mutants' susceptibility to virulent and avirulent *Pseudomonas syringae* pv *tomato*, assessing HR intensity in the suppressor mutants, quantifying *PR1* expression after pathogen infection, and characterising the developmental phenotype of the suppressor mutants. Further experiments will depend on the suppressor mutants identified.

Chapter 2

Material and Methods

2.1 *Arabidopsis thaliana* growth

All plants were *Arabidopsis thaliana* ecotype Columbia (Col-0) unless otherwise indicated [Table 2.1]. Plants were grown in peat moss, vermiculite and sand (4:1:1) at 21°C in long days (16 hours light, 8 hours dark), with 100 μmol m^2 s^{-1} light intensity and 65% humidity.

For growth on plates, seeds were surface sterilised before placing them on plates. Seeds were soaked in water for 30 min, then sterilised with ethanol for 5 min, followed by 1:10 diluted bleach for 5 min. Seeds were then washed with sterile water 3 to 5 times and were then kept in 0.1 % agarose. The seeds were vernalised for 2 days at 4°C before putting them on 1/2 MS (2.2 g/l MS salt, 1 % (w/v) sucrose and 0.8 % (w/v) agar) plates.

Arabidopsis wild type and transgenic lines

Line	Gene(s)	Phenotype/Function	Source
Col-0		wild type	NASC
par2-1	*At5g43940*	*GSNOR1* point mutation High levels of protein S-nitrosylation	Jianru Zuo Bejing, China
atgsnor1-3	*At5g43940*	*GSNOR1* T-DNA insert High levels of protein S-nitrosylation	Gary Loake Edinburgh, UK
Ler atgsnor1-3	*At5g43940*	*Ler* with introgressed *atgsnor1-3* High levels of protein S-nitrosylation	Gary Loake Edinburgh, UK
atgsnor1-3 spl7	*At1g20620* *At5g43940*	*CAT3* point mutation *GSNOR1* T-DNA insert	Gary Loake Edinburgh, UK
atgsnor1-3 spl8	*At1g20620* *At5g43940*	*CAT3* point mutation *GSNOR1* T-DNA insert	Gary Loake Edinburgh, UK
spl7	*At1g20620*	*CAT3* point mutation	
spl8	*At1g20620*	*CAT3* point mutation	
cat1	*At1g20630*	*CAT1* T-DNA insert Reduced catalase activity	Ye-Qin Hu Wuhan, China
cat2	*At4g35090*	*CAT2* T-DNA insert Reduced catalase activity	Ulrike Zentgraf Tuebingen, Germany
cat3	*At1g20620*	*CAT3* T-DNA insert Reduced catalase activity	Ulrike Zentgraf Tuebingen, Germany
cat2/3	*At4g35090* *At1g20620*	*CAT2* and *CAT3* T-DNA inserts Reduced catalase activity	Ulrike Zentgraf Tuebingen, Germany
vtc2-1	*At4g26850*	Reduced ascorbate levels	Nick Smirnoff Exeter, UK
pad2	*At4g23100*	Reduced glutathione levels	Gary Loake Edinburgh, UK
trx3/5	*At5g42980* *At1g45145*	*TRX3* and *TRX5* T-DNA inserts	Steven Spoel Edinburgh, UK
atgsnor1-3 cat1	*At1g20630* *At5g43940*	Reduced catalase activity High levels of protein S-nitrosylation	
atgsnor1-3 cat2	*At4g35090* *At5g43940*	Reduced catalase activity High levels of protein S-nitrosylation	
atgsnor1-3 cat3	*At1g20620* *At5g43940*	Reduced catalase activity High levels of protein S-nitrosylation	
atgsnor1-3 cat2/3	*At4g35090* *At1g20620* *At5g43940*	Reduced catalase activity High levels of protein S-nitrosylation	
atgsnor1-3 pad2	*At4g23100* *At5g43940*	Reduced glutathione levels High levels of protein S-nitrosylation	
atgsnor1-3 trx3	*At5g42980* *At5g43940*	*TRX3* T-DNA insert High levels of protein S-nitrosylation	
atgsnor1-3 trx5	*At1g45145* *At5g43940*	*TRX5* T-DNA insert High levels of protein S-nitrosylation	

Table 2.1: *Arabidopsis* wild type and transgenic lines. Plant lines where no source is given were obtained by crossing.

2.2 Growth and inoculation of *Pseudomonas syringae* pv *tomato* DC3000

Pseudomonas syringae pv *tomato* DC3000, *Pst* DC3000 (*avrB*), *Pst* DC3000 (*avrRps4*) and *Pst* DC3000 (*avrRpm1*) were grown in LB liquid medium (tryptone 10g/l, yeast extract (Oxoid) 5 g/l, NaCl (VWR, UK) 10 g/l) with 50μg/ml rifampicin (supplemented with 50μg/ml kanamycin for *avrB*, *avrRps4* and *avrRpm1*) at 28°C overnight. Cells were harvested at OD_{600} equal to 0.2 (the equivalent of 10^8 colonies ml^{-1}) and pelleted by centrifugation before resuspension in 10 mM $MgCl_2$. Four week old plants were infiltrated with a *Pst* DC3000 suspension (OD_{600}= 0.0002 for resistance assay) or with avirulent *Pst* DC3000 carrying *avrB*, *avrRpm1* or *avrRps4* (OD_{600}= 0.002 for resistance assay, OD_{600}= 0.02 for Trypan Blue staining, and OD600= 0.2 for electrolyte leakage) on the abaxial side of the leaf using a 1 ml syringe [Grant and Loake, 2000].

2.3 *Pst* DC3000 resistance assay

Pst DC3000 was inoculated into plants as described above. Leaves were harvested zero, three and five days post inoculation (dpi) for analysis of bacterial growth. 1 leaf disc (1 cm^2) per plant was collected and up to 16 plants per line were used. Leaf discs were collected in 96-well collection boxes (Qiagen). A single steel ball (3 mm diameter) was added to each tube and the leaf discs were ground up in 500 μl 10 mM $MgSO_4$ using a tissue lyser (Qiagen/Retsch)

for 2 min at 30 shakes per second. 200 μl of the bacterial suspension was transferred to a 96-well plate, and serial dilutions were made to 10^{-4} using a multichannel pipette. Then the multichannel pipette was used to plate 10 μl of each dilution together in stripes onto NYG plates (Bacto peptone 5 g/l, yeast extract (Oxoid) 3 g/l, glycerol (Fisher Scientific) 20 ml/l, Bacto agar 15 g/l) containing the appropriate antibiotics as above. The plates were incubated for 2 days at 28°C and the number of bacterial colonies for each sample was counted and recorded at the best countable dilution.

2.4 DAB staining

One half of each leaf was inoculated with *Pst* DC3000 as described above. Leaves were harvested after 3, 6, 9 and 12 hours and stained with diaminobenzidine (DAB) to visualise H_2O_2 production. The staining solution was prepared by dissolving 1 g/l DAB in boiling water, which was then cooled down to room temperature before being using for staining. The leaves were placed in DAB solution for several hours, usually overnight, and then destained by boiling in 96% ethanol for 10 mins. After destaining, the leaves were washed with dH$_2$O twice and then mounted on microscopic slides.

2.5 Trypan Blue staining

One half of each leaf was inoculated with *Pst* DC3000 as described above. Leaves were harvested after 24 hours and stained with Trypan Blue to visualise

cell death. The staining solution consisted of 25% (v/v) H_2O, 25% (v/v) lactic acid, 25% (v/v) phenol, 25% (v/v) glycerol, and 0.25 g/l Trypan Blue. Leaves were placed in staining solution and boiled for 2 min. The staining solution was allowed to cool down and the leaves were then placed in destaining solution (2.5% (w/v) chloral hydrate) overnight or until destained. The leaves were then mounted on microscopic slides and photographed. The images were analysed using Adobe Photoshop. The inverse luminosity of a square area of the uninoculated and of the inculated half of the leaf was measured, and the value for the uninculated half was substracted from the value for the inoculated half. The uninoculated half of the leaf served as a control, to account for differences in staining.

2.6 Electrolyte Leakage

Pst DC3000 was inoculated into plants as described above. Leaf disks (1 cm in diameter) were collected immediately after inoculation and 10 leaf disks were placed in small petri dishes containing 6 ml of dH_2O. Conductivity was measured 0, 2, 4, 6, 8, and 24 hours post inoculation (DiST WP conductivity meter, Hanna Instruments).

2.7 DNA extraction

DNA was extracted using cetyltrimethylammonium bromide (CTAB) extraction buffer (100 mM Tris HCl, pH 8; 1.4 M NaCl (VWR, UK); 20 mM EDTA;

1% CTAB). For small numbers of samples, DNA extraction was done in Eppendorf tubes and for large numbers of samples, DNA extraction was done in 96-well boxes.

2.7.1 DNA extraction (Eppendorf tubes)

Leaves were collected in 1.5 ml Eppendorf tubes. 300 μl of CTAB buffer was added to each sample and the leaves were ground up using a micropestle. The samples were then incubated at 65°C for at least 10 minutes up to several hours. After letting the samples cool down to room temperature, 300 μl of chloroform were added to each sample. The samples were centrifuged at 15,000 rpm (IEC MicroMax Microcentrifuge) for 2 min to separate the phases. After centrifugation, the aqueous phase (ca. 200 μl) was transferred to a fresh Eppendorf tube containing 200 μl isopropanol. The samples were centrifuged at 15,000 rpm for 5 minutes to pellet the DNA. The supernatant was discarded and the pellets were washed with 70% ethanol by centrifuging at 15,000 rpm for 2 minutes. The ethanol was poured off and the pellets were air dried in a laminar flow hood. The pellets were resuspended in 100 μl dH$_2$O overnight. The DNA was stored at -20°C.

2.7.2 DNA extraction (96-well format)

Leaves were collected in 96-well collection boxes (Qiagen). A single steel ball (3 mm diameter) was added to each tube. The boxes were placed at -70°C for at least 1 hour to freeze the samples. The frozen samples were ground

using a tissue lyser (Qiagen/Retsch) for 2 minutes at 30 shakes per second. The ground tissue was centrifuged briefly (IEC MicroMax Microcentrifuge) to bring down the tissue dust. 300 μl of CTAB buffer was added to each sample using a multichannel pipette. The samples were then incubated in a waterbath at 65°C for at least 30 minutes. After letting the samples cool down to room temperature, 300 μl of chloroform was added to each sample. The boxes were shaken to mix CTAB buffer and chloroform, and then centrifuged at 3250 rpm for 15 minutes to separate the phases. After centrifugation, the aqueous phase (ca. 200 μl) was added to new tubes containing 200 μl isopropanol. The samples were incubated at room temperature for 10 minutes and then centrifuged at 3250 rpm for 15 minutes to pellet the DNA. The supernatant was discarded and the pellets were washed with 70% ethanol by centrifuging at 3250 rpm for 10 minutes. The ethanol was poured off and the pellets were air dried in a laminar flow hood. The pellets were resuspended in 100 μl dH$_2$O overnight. The DNA was stored at -20°C.

2.8 RNA extraction

Total RNA was extracted from 4 week old plant leaves using Tri Reagent (Sigma-Aldrich). Leaves were collected in 1.5 ml Eppendorf tubes, snap frozen in liquid nitrogen and stored at -80 °C until they were used for RNA extraction. The tissue was ground in liquid nitrogen using a pestle and mortar and then transferred to a cold Eppendorf tube. 1 ml of Tri Reagent was added immediately and mixed with the ground tissue by shaking for several seconds. Samples were incubated at room temperature for 5 minutes, then 200 μl of

chloroform was added and samples were vortexed for 15 seconds. Samples were incubated at room temperature for 2-3 mins and centrifuged at 12,000 g for 15 mins at 4°C (IEC MicroMax Microcentrifuge, placed in cold room) to separate the phases. After centrifugation, the aqueous phase (ca. 500 μl) was transferred to a new tube containing 500 μl of 24:1 chloroform:isoamyl alcohol. Samples were vortexed for 30 seconds and then centrifuged at 12,000 g for 5 minutes at 4°C to separate the phases. The aqueous phase was transferred to a new tube containing 50 μl of 3M sodium acetate pH 5.2 and 1 ml of 100% ethanol. Samples were mixed by inverting the tube and then placed at -20°C for at least 1 hour to precipitate the RNA. The samples were centrifuged at 14,000 rpm for 15 minutes at 4°C to pellet the RNA. The supernatant was discarded and the pellets were washed twice with 70% ethanol by centrifuging at 14,000 rpm for 5 mins at 4°C. The supernatant was poured off and tubes were centrifuged briefly to bring down residual fluid, which was then removed using a pipette. Pellets were air dried in a laminar flow hood for 10-15 mins and then resuspended in 10 μl dH$_2$O. The RNA was stored either at -20°C (short term) or at -70°C (long term).

2.9   PCR based methods

All primers were from Sigma-Aldrich, UK. PCR reactions were run on a PTC-200 Peltier Thermal Cycler. The detailed list of primers and their sequences can be found in appendices 1-5.

2.9.1   Map-based cloning

The principles of map-based cloning are described in more detail in chapter 4.
DNA from the mapping population was extracted using CTAB as described
above.   10 InDel markers were used for rough mapping [Appendix 1].   All
InDel markers used for rough mapping, except K11J9, were as previously de-
scribed [Zhang et al., 2007b]. SSLP and InDel markers [Appendix 2], as well
as SNAP markers [Appendix 3], were used for fine mapping.   SLLP mark-
ers were obtained from the TAIR website [`http://www.arabidopsis.org/`].
InDel markers used for fine mapping were designed from the Monsanto *Ara-
bidopsis* polymorphism and *Ler* sequence collections [Jander et al., 2002], using
Primer3 [`http://frodo.wi.mit.edu/primer3/`] [Rozen and Skaletsky, 1999].
SNPs were identified using the Monsanto *Arabidopsis* polymorphism and *Ler*
sequence collections, and SNAP markers were designed using WebSNAPER
[`http://ausubellab.mgh.harvard.edu/`] [Drenkard et al., 2000].   Cycling
conditions for SSLP and InDel markers were 94°C for 2 min, then 40 times
(94°C for 15 sec, 55°C for 30sec, 68°C for 1 min), then 68°C for 7 min. Cycling
conditions for SNAP markers were 94°C for 5 min, then 28 times (2 °C/sec to
94°C, 94°C for 30 sec, 1.4°C/sec to 64°C, 64°C for 1 min), then 72°C for 10
min. All SSLP markers and the InDel markers F19K19, T13M22, T20H2 and
T22I11 were run on 3% (w/v) high resolution agarose gels; all other markers
were run on 1% (w/v) agarose gels. Col-0 and *Ler* DNA samples were included
on each gel as controls, and a 1 kb and/or 100 bp DNA ladder (Promega, UK)
were also included on each gel to determine the sizes of the PCR products.
Once the location of the mutation was narrowed down to a 60 kb region, all

genes in this area were sequenced to identify the mutation [Sorhagen, 2010].

2.9.2   Genotyping of T-DNA insertion lines

Two gene-specific primers were used together with a left border primer for the T-DNA [Appendix 4], resulting in a larger band for the wild-type gene and a smaller band for the T-DNA, with the exception of *trx5*, where the T-DNA band is larger. Primers were designed using the T-DNA Express website [`http://signal.salk.edu/tdnaprimers.2.html`]. Primer sequences for genotyping *cat2* and *cat3* were provided by Ulrike Zentgraf, Tuebingen. PCR reactions were mixed in 25 μl volumes and consisted of 2.5 μl home made 10x PCR buffer, 0.5 μl 10mM (each) dNTPs, 2.5 μl 25mM $MgCl_2$, 1 μl 10 μM forward primer, 1 μl 10 μM reverse primer, 1 μl 10 μM left border primer and 15.4 μl ddH_2O, 1 μl DNA and 0.1 μl home made Taq polymerase (5 u/μl). In some cases it was not possible to use all three primers in the same PCR reaction, and two separate PCR reactions were performed, one with forward and reverse primers, and one with left border and reverse primers. Cycling conditions for genotyping *atgsnor1-3* were 94°C for 1 min, then 35 times (94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min), then 72°C for 7 min. Cycling conditions for genotyping *cat2* were 94°C for 1 min, then 35 times (94°C for 30 sec, 70°C for 30 sec, 72°C for 1 min), then 72°C for 7 min. Cycling conditions for genotyping all other T-DNA lines were 94°C for 1 min, then 35 times (94°C for 30 sec, 58°C for 30 sec, 72°C for 1 min), then 72°C for 7 min. PCR products were run on 1% (w/v) agarose gels and stained with ethidium bromide.

2.9.3 Genotyping of point mutation lines

vtc2-1 plants were genotyped using a CAPS (cleaved amplified polymorphic sequence) marker as previously described [Dowdle et al., 2007]. The *vtc2-1* mutation introduces a HindIII restriction site. PCR amplification using two flanking primers yields a a 767 bp amplicon, and a subsequent restriction digest with HindIII results in 588 and 179 bp fragments if the *vtc2-1* mutation is present. The cycling conditions for genotyping *vtc2-1* were 94°C for 1 min, then 35 times (94°C for 30 sec, 58°C for 30 sec, 72°C for 1 min), then 72°C for 7 min. All other point mutation lines were genotyped using SNAP markers [Appendix 5], which were designed using WebSNAPER [`http://ausubellab.mgh.harvard.edu/`] [Drenkard et al., 2000]. Cycling conditions for SNAP markers were 94°C for 5 min, then 28 times (2 °C/sec to 94°C, 94°C for 30 sec, 1.4°C/sec to 64°C, 64°C for 1 min), then 72°C for 10 min. PCR products were run on 1% (w/v) agarose gels and stained with ethidium bromide.

2.9.4 RT-PCR

Reverse transcription (total volume 10 μl) was carried out using the OmniScript kit (Qiagen, CA) according to the manufacturer's instructions. 1 μl of this reaction was then used as a template for semiquantitative RT-PCR (total volume 25 μl). The cycling conditions for RT-PCR were 94°C for 1 min, then 30 times (94°C for 40 sec, 55°C for 40 sec, 72°C for 1 min), then 72°C for 7 min. PCR products were run on 1% (w/v) agarose gels and stained with ethidium bromide.

2.10   Transgenic line construction

atgsnor1-3 spl7 and *atgsnor1-3 spl8* were obtained by mutagenising *atgsnor1-3* seeds with ethyl methane sulfonate (EMS) and then selecting plants that showed wildtype phenotype [Sorhagen, 2010]. *spl7* and *spl8* were generated by crossing *atgsnor1-3 spl7* and *atgsnor1-3 spl8* with Col-0 and selecting recombinants with genotyping PCR as above.

To generate the *2x35S::CAT3 atgsnor1-3 spl8* line, the wildtype *CAT3* gene was cloned into a vector with a constitutive promoter (pGreen 0229-2x35S) [Hellens et al., 2000] using standard molecular biology techniques [Sambrook et al., 2001]. *spl7 gsnor1-3* and *spl8 gsnor1-3* plants were transformed by floral dipping [Clough and Bent, 1998]. Transgenic plants were selected using the herbicide Basta (150 μg/ml).

atgsnor1-3 plants were crossed with *cat1*, *cat2*, *cat3*, *cat2/3*, *pad2*, *vtc2-1*, and *trx3/5* plants to generate double or triple knock-out mutants. Recombinants were selected with genotyping PCR as above.

2.11   Catalase activity assay

Catalase activity was measured as described previously [Contento and Bassham, 2010] with some changes made to the protocol. For each sample, 200 mg leaf tissue were collected from four to six different plants of the same age and ground in chilled extraction buffer (100 mM potassium phosphate buffer, pH 6.5; cOmplete, EDTA-free protease inhibitor (Roche)). The samples were cen-

trifuged at 10,000 g for 15 min at 4°C (IEC MicroMax Microcentrifuge, placed in cold room). A 25 μl sample of the supernatant was added to 975 μl of 100 mM potassium phosphate buffer (pH 6.5) containing 5 mM H_2O_2 and the change in A_{240} was measured for 3 minutes. The rate of decomposition of H_2O_2 by catalase was calculated using a micromolar extinction coefficient of 0.0436 cm^2/μmol. Total protein was measured using a Bradford assay, according to the manufacturer's instructions (BioRad, Hercules, CA).

Chapter 3

Isolation of mutants from an

atgsnor1-3 suppressor screen

3.1 The model plant *Arabidopsis thaliana*

The small mustard weed *Arabidopsis thaliana* (family *Brassicaceae*), commonly known as thale cress, is a very important model organism in plant sciences [Meinke et al., 1998]. Friedrich Laibach proposed its use as a plant model system as early as 1943 [Laibach, 1943]. *Arabidopsis* is ideally suited as a model plant because of its small size, its fast generation time of only 6 weeks under ideal growth conditions, its high seed yield of up to 10,000 seeds per plant, and its ability to grow well in either soil or in media under controlled conditions [Page and Grossniklaus, 2002]. *Arabidopsis* plants are self-fertilising but can also be out-crossed if necessary. *Arabidopsis* is diploid and has the smallest known plant genome (125 Mb), consisting of

five chromosomes with 25,498 genes [Arabidopsis Genome Initiative, 2000], and plants can easily be transformed, for example through the floral dip method [Clough and Bent, 1998]. The whole genome and other tools can be freely accessed through The *Arabidopsis* Information Resource (TAIR) website [`http://www.arabidopsis.org/`].

3.2 Genetic screens

Sequencing of the whole *Arabidopsis* genome [Arabidopsis Genome Initiative, 2000] was an important milestone. However, the functions of many genes and regulatory sequences are still unknown, so a lot remains to be done. Gene functions can be characterised through genetic screens, either reverse genetic screens or forward genetic screens. In reverse genetic screens a known gene is disrupted to investigate its function, whereas in forward genetic screens the starting point is a mutant phenotype caused by an unknown mutation.

Agrobacterium-mediated transformation can be used to stably integrate T-DNA into the *Arabidopsis* genome, which often leads to disruption of a gene. T-DNA insertion lines are very useful for reverse genetics and over 225,000 T-DNA insertion lines are available which cover almost the entire *Arabidopsis* genome [Alonso et al., 2003]. In forward genetic screens, plants are screened for the desired mutant phenotype and then the underlying mutation is identified via map-based cloning or next generation sequencing [Page and Grossniklaus, 2002]. Mutations can be artificially induced, mainly through ethylmethane sulphonate (EMS) but also through ionising ratiation, or could

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be due to natural variation in wild type populations. Second-site modifier screens are used to screen for mutations that either enhance or suppress the first mutation, and can lead to the discovery of genes that act redundantly with the first mutation or that are involved in alternative pathways [Page and Grossniklaus, 2002].

3.3   Isolation of *atgsnor1-3* suppressor mutants

A suppressor screen was used to identify suppressors of *atgsnor1-3*. The aim of such a screen is to find a second mutation that suppresses the effects of a first mutation. In *Arabidopsis*, suppressor screens have been used to analyse growth signalling pathways, such as ABA and gibberellin pathways [Steber et al., 1998, Peng et al., 1999] and also to investigate pathways involved in disease signalling [Li et al., 1999] and stress responses [Wagner et al., 2004].

atgsnor1-3 seeds were mutagenised with EMS [Sorhagen, 2010], which is an alkylating agent and introduces random point mutations, usually from G/C to A/T [Greene et al., 2003]. *atgsnor1-3* plants have increased SNO levels, reduced SA accumulation, reduced and delayed *PR1* expression, disease susceptibility and altered morphological phenotype [Feechan et al., 2005]. The *atgsnor1-3* mutant [Fig. 3.1B] has lost apical dominance, develops a high number of lateral shoots and exhibits stunted growth. This distinct morphology makes it fairly easy to identify putative suppressor mutants. In order to find suppressors of *atgsnor1-3*, a population of EMS mutagenised plants was screened for plants that showed wild type shoot morphology [Sorhagen,

2010]. It was originally speculated that suppressor mutations would result in wild-type SNO levels, so they were called 'SNOplough' (*spl*). Two mutants with wild type shoot morphology, *atgsnor1-3 spl7* [Fig. 3.1C] and *atgsnor1-3 spl8* [Fig. 3.1D], were identified in the screen of the recessive population (M_2 plants) [Sorhagen, 2010], indicating that they are most likely recessive mutations.

Both mutants were backcrossed with the background *atgsnor1-3* mutant to confirm that *spl7* and *spl8* were recessive mutations and to remove unrelated mutations [Sorhagen, 2010]. All F_1 plants exhibited an *atgsnor1-3* phenotype and the F_2 plants showed a 1:3 segregation ratio as would be expected for a single recessive gene mutation [Sorhagen, 2010]. To test if the mutations are allelic, the two mutants were crossed with each other. The F_1 plants [Fig. 3.1E] had the same morphological phenotype as the parents, indicating that the mutations are allelic [Sorhagen, 2010].
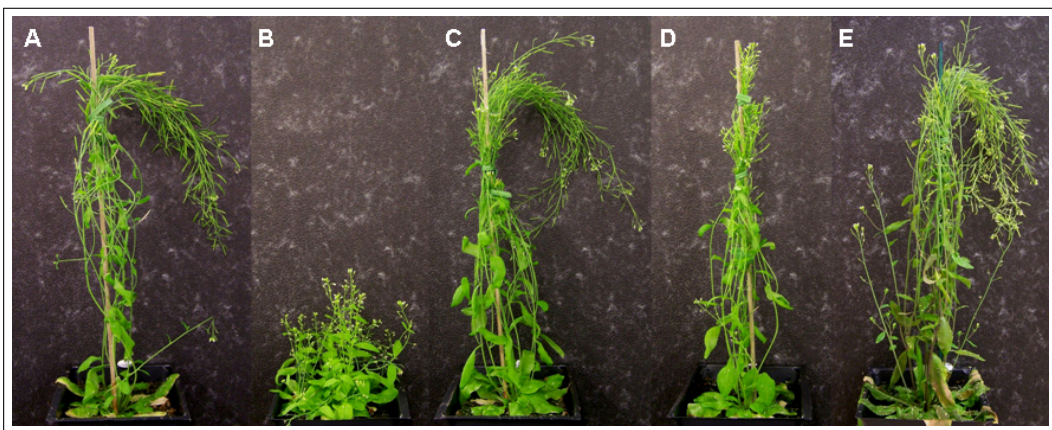
Figure 3.1: 9 week old *Arabidopsis* plants. (A) Col-0 (wild type), (B) *atgsnor1-3*, (C) *atgsnor1-3 spl7*, (D) *atgsnor1-3 spl8*, (E) *atgsnor1-3 spl7* x *atgsnor1-3 spl8* F_1 [pictures taken by Kirsti Sorhagen]

3.4 Phenotype of the suppressor mutants

Both *atgsnor1-3 spl7* and *atgsnor1-3 spl8* seemed to exhibit wild type growth morphology. However, closer examination revealed that the *spl7* and *spl8* mutations only partially suppressed the *atgsnor1-3* phenotype. While the suppressor mutants have restored apical dominance, they did not reach the same height as Col-0 and appeared to be slightly bushier, particularly in the case of *atgsnor1-3 spl8* [Fig. 3.2].

The suppressor mutants have restored apical dominance. Both *atgsnor1-3 spl7* and *atgsnor1-3 spl8* showed the same number of first order lateral branches as Col-0, whereas *atgsnor1-3* had a very high number of first order lateral branches, resulting in bushy growth [Adil Hussain, unpublished results]. The number of floral nodes in *atsgnor1-3 spl7* and *atgsnor1-3 spl8* was also very similar to Col-0, while it was very high in *atgsnor1-3* [Adil Hussain, unpublished results]. Both suppressor mutants only reached about two thirds of the height of Col-0 plants [Fig. 3.3]. There was no difference in the number of rosette leaves between the suppressor mutants and either Col-0 or *atgsnor1-3* [Adil Hussain, unpublished results]. The hypocotyl lengths of 10 day old suppressor mutants were very similar to Col-0, whereas *atgsnor1-3* hypocotyls were shorter [Adil Hussain, unpublished results]. There were striking differences in leaf area and root length between the suppressor mutants, Col-0 and *atgsnor1-3*. Col-0 rosette leaves were smaller than *atgsnor1-3* leaves, whereas the leaf area of both suppressor mutants was bigger than that of *atgsnor1-3* [Adil Hussain, unpublished results]. The opposite pattern was observed for root length, *atgsnor1-3* and the suppressor mutants had significantly shorter
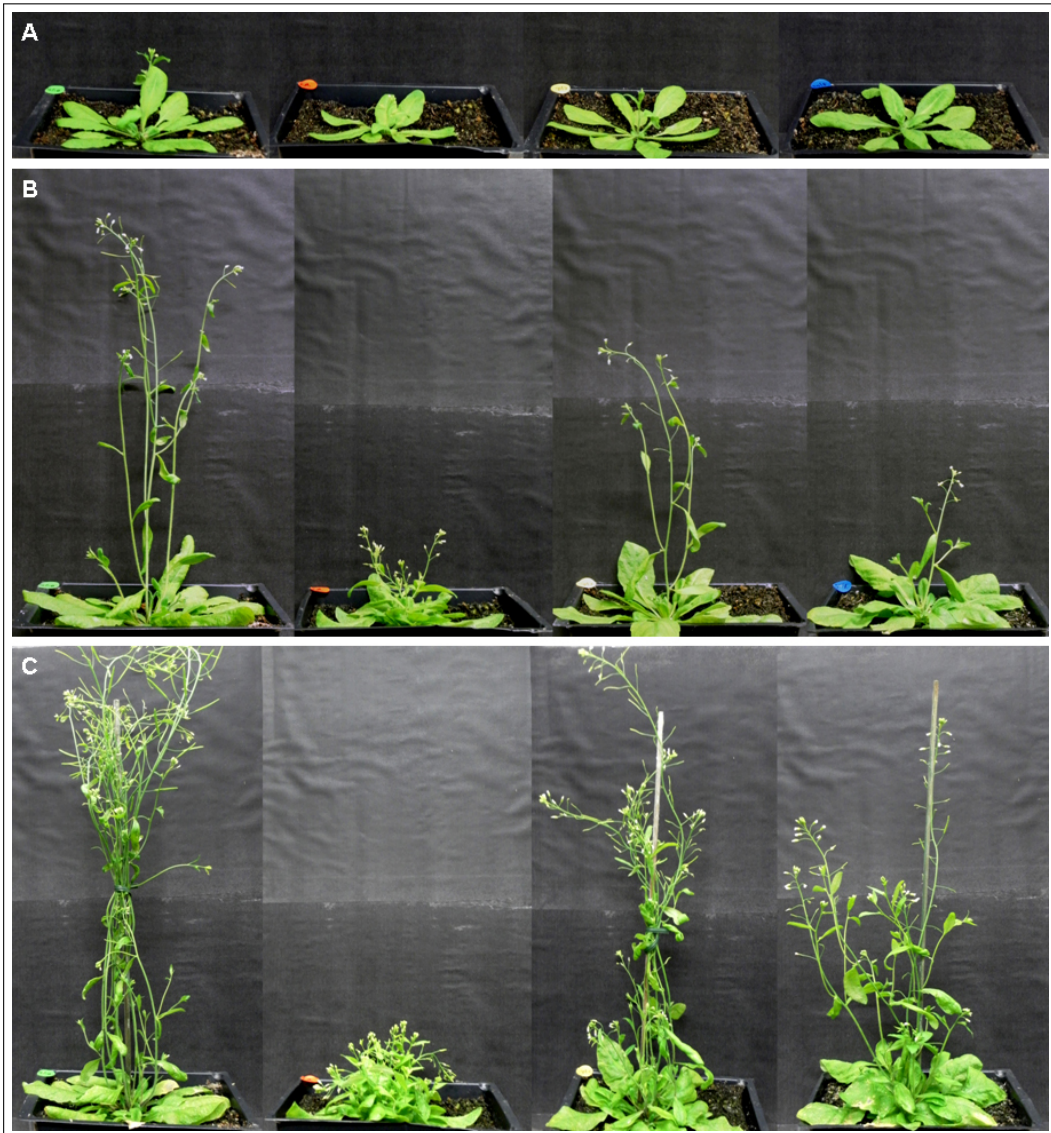
Figure 3.2: Growth of suppressor mutants compared to Col-0 and *atgsnor1-3*. From left to right: Col-0 (wild type), *atgsnor1-3*, *atgsnor1-3 spl7*, *atgsnor1-3 spl8*. (A) 5 week old plants. (B) 6 week old plants. (C) 7 week old plants.

roots than Col-0 [Fig. 3.4]. The siliques of both suppressor mutants were considerably smaller than Col-0 siliques and roughly the same size as *atgsnor1-3* siliques. The siliques of *atgsnor1-3* and the suppressor mutants contained about half to two thirds the number of seeds of Col-0 siliques, and there was
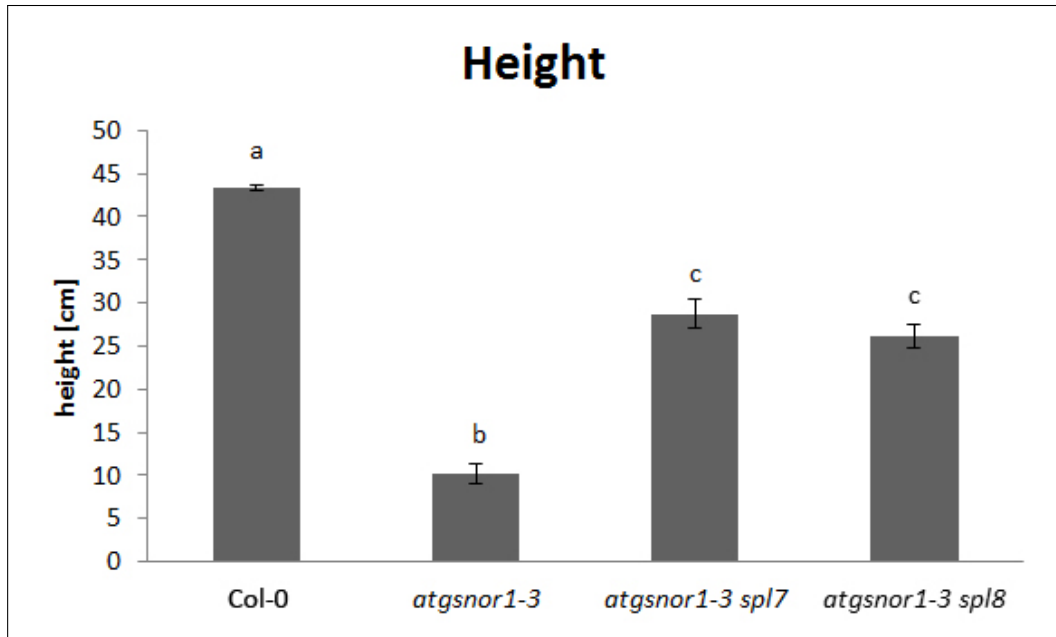
Figure 3.3: Height (cm) of 7 week old plants. Error bars show SE (n=6; n=5 for Col-0). Values with different letters are significantly different (P<0.01, Duncan's MRT).
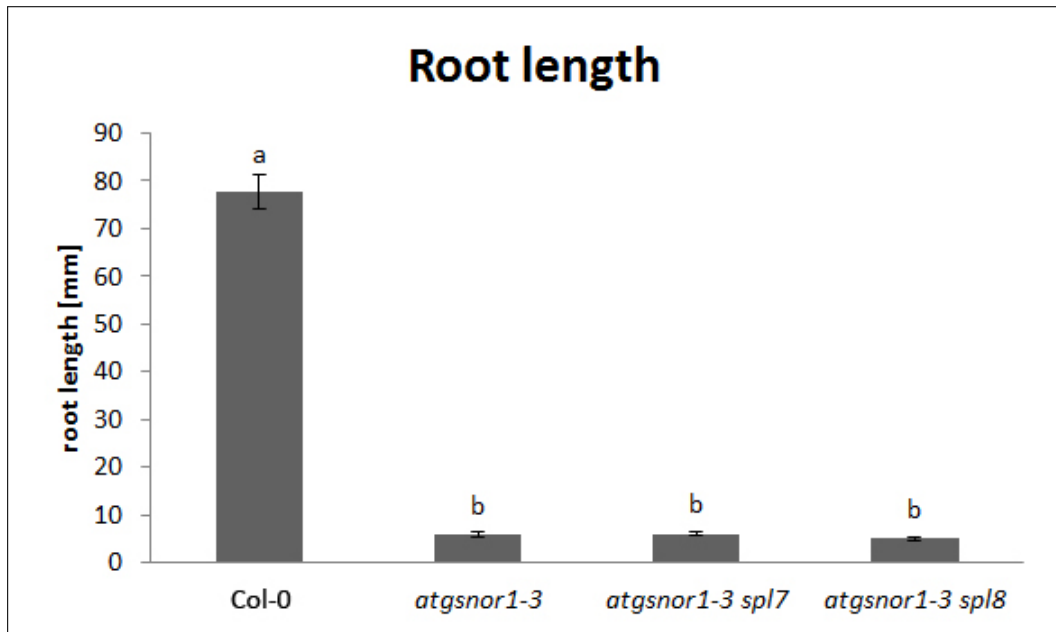
Figure 3.4: Root lengths (mm) of 2 week old plants. Error bars show SE. Values with different letters are significantly different (P<0.01, Duncan's MRT).
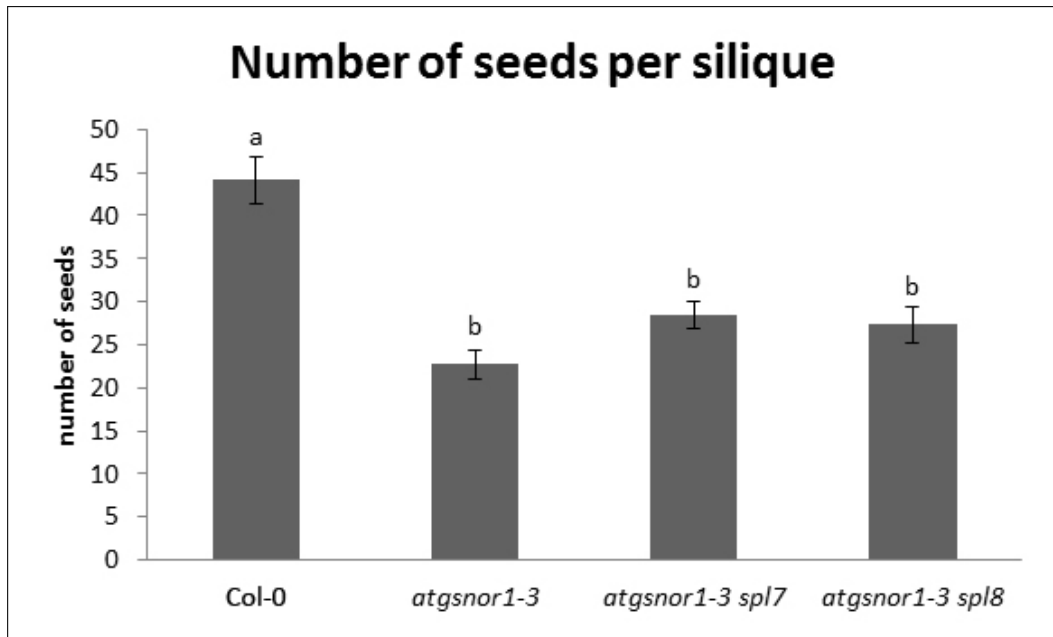
Figure 3.5: Number of seeds per silique. Error bars show SE (n=20). Values with different letters are significantly different (P<0.01, Duncan's MRT).
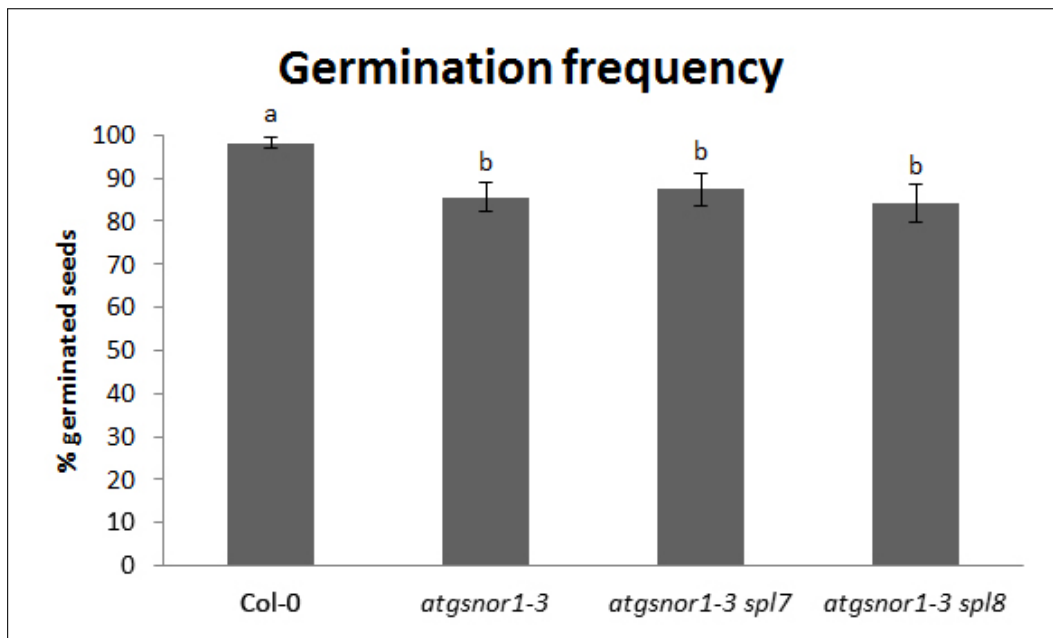
Figure 3.6: Germination frequency (out of 10 seeds). Error bars show SE (n=34).Values with different letters are significantly different (P<0.01, Duncan's MRT).

no significant difference between the number of seeds per silique in *atgsnor1-3* and the suppressor mutants [Fig. 3.5]. Col-0 showed a germination frequency of almost 100%, while only about 85% of the *atgsnor1-3* and suppressor mutant seeds germinated [Fig. 3.6].

3.5 Discussion

An EMS suppressor screen of *atgsnor1-3* resulted in the identification of two recessive and allelic mutants, *atgsnor1-3 spl7* and *atgsnor1-3 spl8*, which had recovered apical dominance. However, other aspects of the phenotype were still unchanged, so the *spl7* and *spl8* mutations appear to only partially suppress the *atgsnor1-3* phenotype. Only two allelic suppressor mutants were isolated from the screen, which is a very low number.

In this suppressor screen, 35,000 M_1 seeds were mutagenised using 0.3% (w/v) EMS, which resulted in 137,000 M_2 plants [Sorhagen, 2010]. In one study it has been suggested that mutagenising 45,000 seeds using 0.13% - 0.25% (w/v) EMS is sufficient to achieve 95% genome coverage [Jander et al., 2003], while in another case it was recommended to mutagenise 125,000 seeds with 0.4% EMS [Kim et al., 2006].

Going by these aforementioned two studies, it is quite possible that an insufficient number of seeds was used in the suppressor screen and that not all possible suppressor mutants were uncovered. However, it is quite intriguing that the only two suppressor mutants that were identified are allelic. This points towards another possible explanation, that there are no other point

mutations that would lead to a suppression of the *atgsnor1-3* developmental phenotype.

Another possible problem is the screening procedure that was used. The suppressor screen utilised wild-type shoot morphology, which has the advantage that it is relatively easy to score, but there is a danger of identifying mutations involved in development and not in SNO regulation. A better option would have been to screen mutagenised seeds on GSNO plates. *atgsnor1-3* plants are significantly more sensitive to GSNO than wild type plants [Sorhagen, 2010], therefore any suppressor mutants involved in SNO regulation would be expected to exhibit increased resistance to GSNO compared to *atgsnor1-3* plants. *atgsnor1-3* plants are also resistant to the superoxide donor paraquat, whereas wild type plants are very sensitive [Chen et al., 2009]. However, using paraquat resistance as a screening method might be problematic because suppressor mutants would be expected to be very sensitive to paraquat and might therefore not be able to survive on paraquat plates. *atgsnor1-3* plants are heat-sensitive [Lee et al., 2008], and have shorter roots and smaller siliques than wild-type plants; assessing loss of heat sensitivity, root or silique length could also be used to screen for suppressor mutants. Another, but more labour-intensive screening option would be to screen for plants that exhibit wild type pathogen resistance.

It is also worth noting that the *spl7* and *spl8* mutations only partially repressed the *atgsnor1-3* developmental phenotype, so it might well be possible that to fully suppress all aspects of the *atgsnor1-3* phenotype only one mutation is not sufficient and several mutations are required.

Auxin has been shown to regulate shoot branching and disruption of polar auxin transport leads to increased branching and loss of apical dominance [Leyser, 2003]. The *atgnsor1-3* mutant shows a phenotype similar to that of mutants impaired in polar auxin transport, such as *bushy and dwarf 1* [Dai et al., 2006] or *transport inhibitor response 3* [Ruegger et al., 1997]. It can be speculated that loss of apical dominance and stunted growth in *atgsnor1-3* is due to a disruption of polar auxin transport and/or auxin signalling, and that the suppressor mutations restore auxin transport and/or signalling.

Chapter 4

Identification of *atgsnor1-3* suppressor mutations

4.1 Principles of map-based cloning

Map-based cloning or positional cloning is used to identify mutations in an unknown position in the genome. Genetic markers with a known location in the genome are used to search for linkage with the unknown mutation, progressively narrowing down the interval until it is small enough to be sequenced [Lukowitz et al., 2000].

Genetic markers are based on polymorphisms which occur between *Arabidopsis* ecotypes or accessions. The most commonly used accessions for map-based cloning are Columbia-0 (Col-0) and *Landsberg erecta* (*Ler*), because they have a high number of known polymorphisms between them. Polymerase chain reactions (PCR) and agarose gel electrophoresis are used to visualise

these polymorphisms.

There are several different types of polymorphisms. As the name suggests, insertions/deletions (InDels) are insertions or deletions between accessions, which have varying length [Zhang et al., 2007b]. Simple sequence repeats (SSRs), also known as Simple sequence length polymorphisms (SSLPs), are repeated sequences of varying length between two accessions. Both InDels and SSLPs can be visualised by PCR with primers flanking the polymorphism, followed by agarose gel electrophoresis [Fig. 4.2]. Single nucleotide polymorphisms (SNPs) are single basepair differences between accessions. One method to visualise SNPs is the use of SNAP (single nucleotide amplified polymorphism) markers, which allow amplification of a PCR product in one accession but not the other [Fig. 4.3] [Drenkard et al., 2000]. A specific set of primers is designed for each accession.

To generate a mapping population, the mutant line is crossed with another accession, usually Col-0 with *Ler*. After the accessions have been crossed, the segregating F_2 population is used to identify plants displaying the mutant phenotype (for recessive mutations) or wild-type phenotype (for dominant mutations) and only these plants are used for mapping [Fig. 4.1]. For mapping of suppressor mutations, both accessions must contain the mutation being suppressed, otherwise the mapping population will not segregate based on the second mutation. Introgression of the original mutation into the other accession can be achieved by crossing the other accession with the mutant line and then backcrossing the other accession with itself [Zhang et al., 2007b]. Seven backcrosses are sufficient to get 99% saturation of the other accession's DNA.
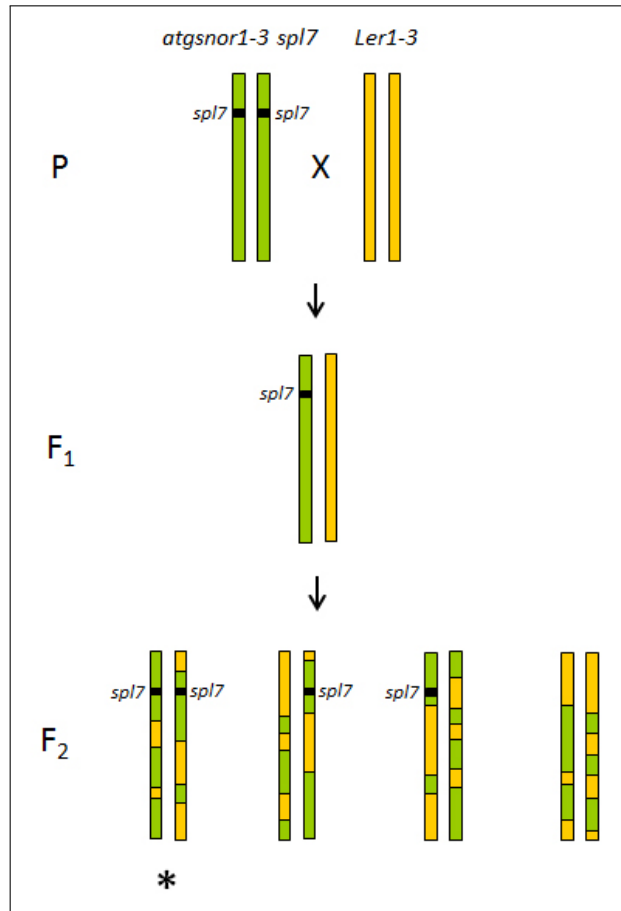
Figure 4.1: Generation of the mapping population for map-based cloning of *spl7*. Only chromosome 1 is shown (green bars represent Col-0 DNA, and yellow bars represent *Ler* DNA). *atgsnor1-3 spl7* is crossed with *Ler1-3*, resulting in a heterozygous F_1 generation. In the segregating F_2 generation approximately 25% of the plants are homozygous for *spl7* (marked with a star), and only these plants are used for map-based cloning while the remaining plants are discarded. Plants homozygous for *spl7* exhibit wild type shoot morphology and can therefore be easily identified.
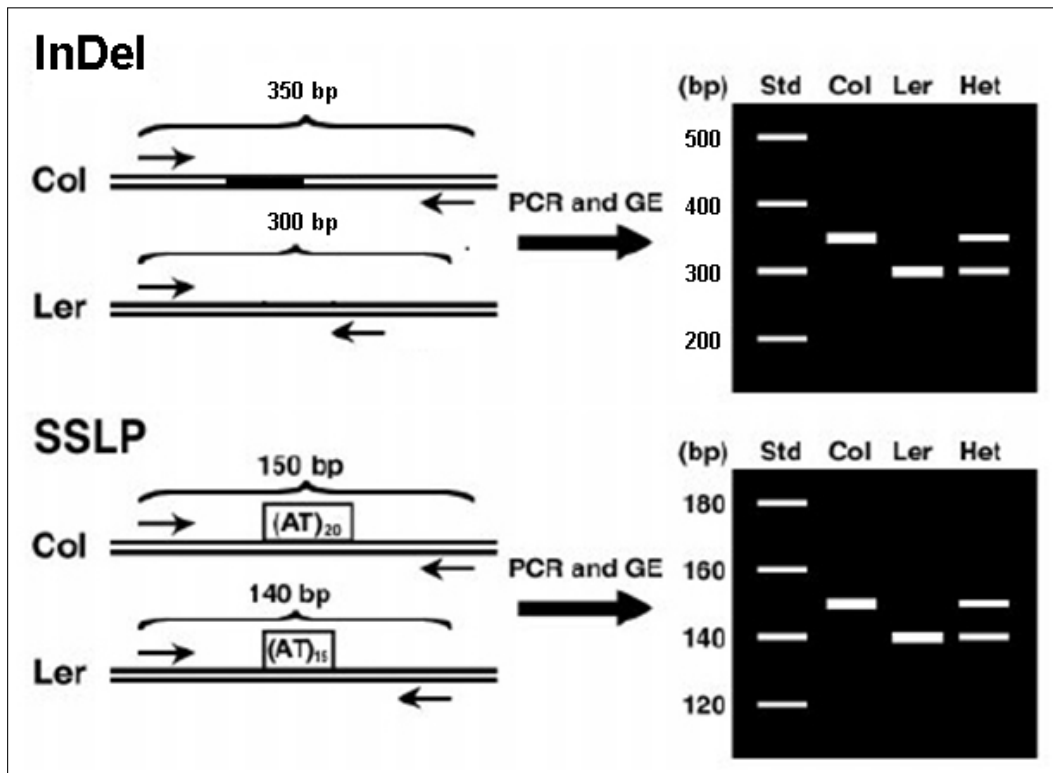
Figure 4.2: InDel and SSLP markers. A primer pair (arrows) is used to amplify a region containing either an insertion/deletion or a short repetive element. In-Del marker: In this example a 50 bp region (shown in black) is present in Col-0 but deleted in *Ler*, resulting in a bigger PCR product in Col-0 compared to *Ler*. The size differences can be visualised by agarose gel electrophoresis (GE) using a standard (Std) as comparison. In plants heterozygous for this polymorphism (Het), both PCR products are amplified, resulting in two bands. SSLP marker: In this example Col-0 has a short repetitive element containing 20 (AT) repeats whereas *Ler* has 15 (AT) repeats, resulting in a Col-0 product of 150 bp and a Ler product of 140 bp. Adapted from [Lukowitz et al., 2000]
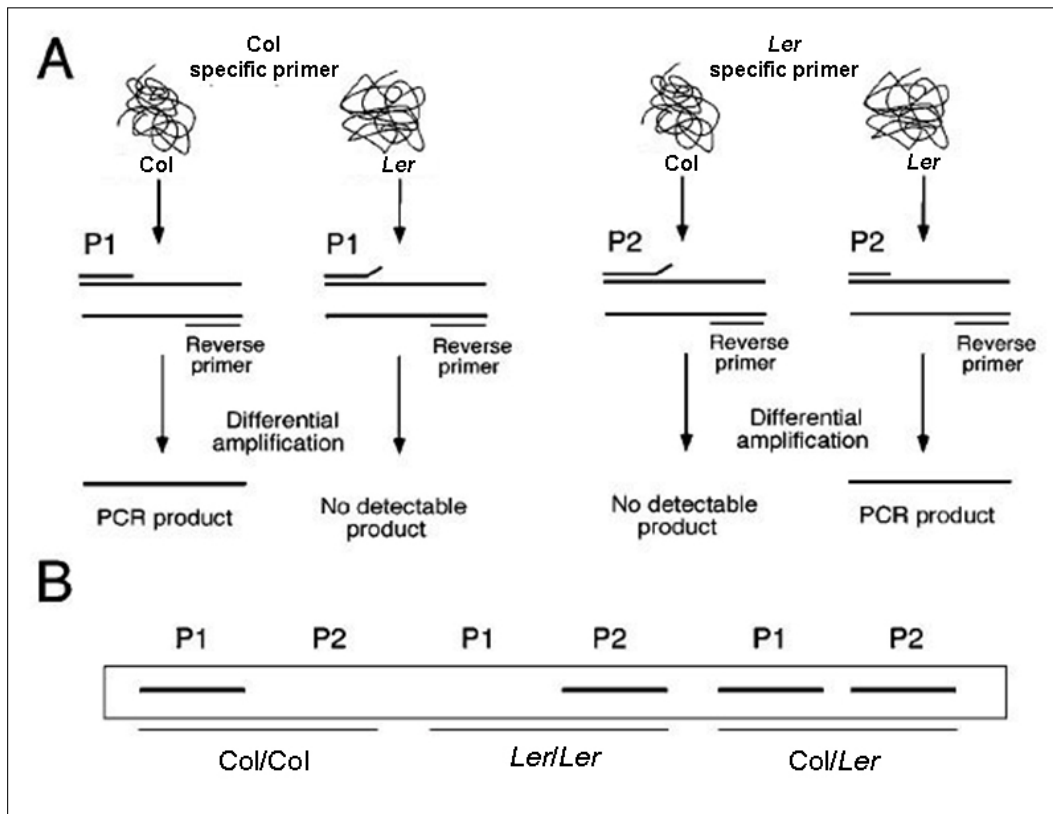
Figure 4.3: SNAP markers. (A) Primers that correspond to a SNP site are used to preferentially amplify specific alleles. Primer pair P1 is an identical match with the Col-0 allele but has a mismatch with the *Ler* allele, whereas primer pair P2 is an identical match with the *Ler* allele but has a mismatch with the Col-0 allele. (B) Schematic representation of agarose gel electrophoresis showing the expected outcome for plants homozygous and heterozygous for both alleles using primer pair P1 and P2. Modified from [Drenkard et al., 2000]

Rough mapping or bulked segregant analysis is first performed on pooled DNA from 25-50 plants to identify a rough chromosomal position of the mutation [Michelmore et al., 1991]. This allows identification of linked markers with a reduced number of PCR reactions. A set of 10 InDel markers, two per chromosome, have been described for rough mapping when Col-0 and *Ler* accessions are used [Fig. 4.4] [Zhang et al., 2007b]. For unlinked markers Col-0

and *Ler* bands of the same intensity would be observed, whereas for linked markers a stronger Col-0 band (if the mutation is in Col-0 background) or stronger *Ler* band (if the mutation is in *Ler* background) would occur.
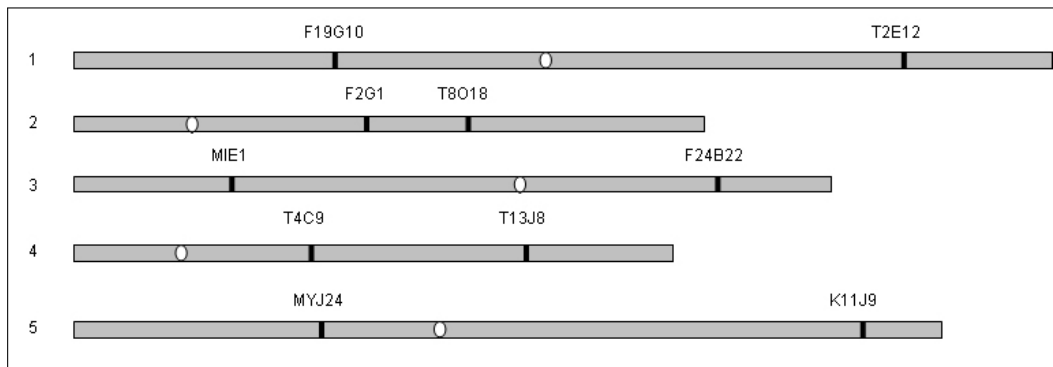
Figure 4.4: Schematic representation of the five Arabidopsis chromosomes showing the position of the 10 InDel markers used for rough mapping. Marker positions are shown as black lines and centromeres are represented by white circles. [Image by Kirsti Sorhagen]

After a linked marker has been identified, flanking markers are used for fine mapping. This involves genotyping a large population to find the recombination frequency (Rf) for each marker. The closer a marker is to a mutation, the lower the Rf will be. A Rf higher than 50% indicates no linkage, whereas a 1% Rf corresponds to a physical distance of approximately 250 kb in *Arabidopsis* [Lukowitz et al., 2000]. The recombination frequency is also dependent on the chromosomal position. Once the mapped region has been narrowed down to about 40 kb or less, sequencing and complementation experiments can be used to identify the mutated gene.

4.2 Rough mapping of *spl7* and *spl8*

The mapping population was generated by crossing the *atgsnor1-3* suppressor mutants with *Ler atgsnor1-3* (introgressed line). The F_1 plants from this cross were allowed to self-fertilise, and then all F_2 plants which showed apical dominance were selected for mapping. The *spl7* and *spl8* mutations are reces-
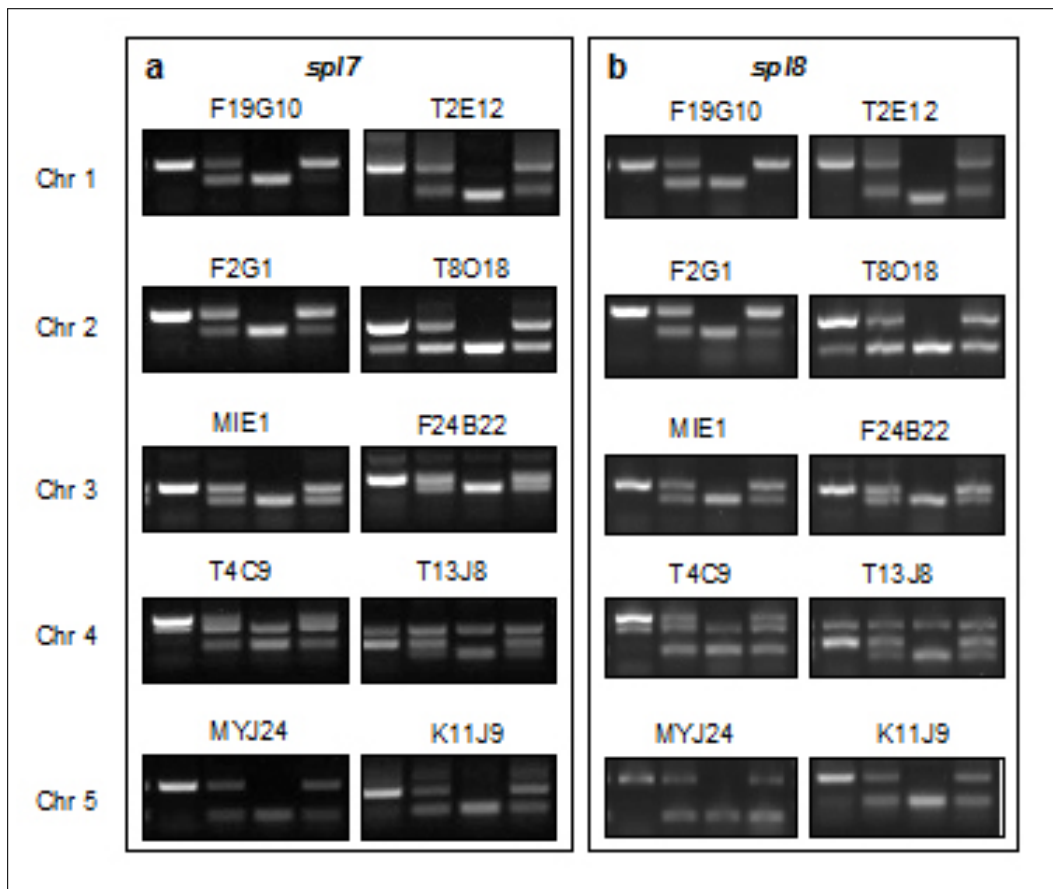
Figure 4.5: Bulked segregant analysis of the *spl7* and *spl8* mutations. Each panel shows from left to right: Col-0 sample, F_1 sample from the mapping cross (heterozygous), *Ler* sample, and the pooled F_2 samples. (a) Rough mapping results for *spl7*. The pooled mutant DNA clearly segregated with marker F19G10 on chromosome 1. (b) Rough mapping results for *spl8*. As expected for allelic mutations, the pooled mutant DNA segregated with the same marker (F19G10) as *spl7*. [Sorhagen, 2010]

sive, so only plants that are homozygous for the mutations would show apical dominance.

Bulked segregant analysis was performed to identify the rough positions of the mutations. DNA from 48 plants for *atgsnor1-3 spl7* and 36 plants for *atgsnor1-3 spl8* was pooled and analysed using ten InDel markers, with two markers on each chromosome [Sorhagen, 2010]. Because the mutations are allelic, they were expected to segregate with the same genetic marker, which was the case. Both mutations gave a strong Col-0 band when PCR was performed with the marker F19G10 which is located on the left arm of chromosome 1 [Fig. 4.5]. Bulked segregant analysis was then performed using four additional markers flanking the marker F19G10, which confirmed that the mutation was indeed located in the area around marker F19G10 [Sorhagen, 2010]. Fine mapping was done for *spl7* only, because there was strong evidence that *spl7* and *spl8* were mutations in the same gene.

4.3   Fine mapping of *spl7*

For fine mapping of *spl7*, a total of 24 mapping markers were used [Table 4.1]. To calculate the Rf for each marker, the number of *Ler* alleles was divided by the total number of alleles [for examples of mapping gels see Fig. 4.6 and Fig. 4.7]. Plants that were heterozygous for all markers were excluded because they had no recombination events in the region of interest and were therefore not useful for calculating the Rf.

Rough mapping showed that the *spl7* mutation segregated with the

Markers used for fine mapping of *spl7*

Marker Name	Type	Location Chr/Mb	Recombinants	Alleles	Rf (%)
F21B7	SSLP	1/0.92	29	148	19.595
Nga63	SSLP	1/3.55	20	148	13.514
F9L1	InDel	1/5.25	10	386	2.591
SRP54A	SSLP	1/5.27	9	384	2.344
T24D18	InDel	1/5.48	9	384	2.344
F19K19	InDel	1/5.71	34	1082	3.142
T13M22	InDel	1/5.92	22	1056	2.083
F25I16	SNAP	1/6.38	43	2012	2.137
T29M8	SNAP	1/6.62	34	2012	1.690
F18O14	SNAP	1/6.75	26	2010	1.294
F14P1	SNAP	1/6.81	22	2010	1.095
T20H2	SNAP	1/6.92	14	2008	0.697
T2OH2	InDel	1/6.98	11	2006	0.548
T20H2-B	**SNAP**	**1/7.00**	**10**	**2010**	**0.498**
F14O10	**SNAP**	**1/7.03**	**9**	**2202**	**0.409**
F14O10-B	**SNAP**	**1/7.06**	**7**	**2200**	**0.318**
F5M15-B	**SNAP**	**1/7.09**	**4**	**2200**	**0.182**
F5M15	**SNAP**	**1/7.11**	**2**	**2202**	**0.091**
F2D10-B	**SNAP**	**1/7.13**	**1**	**2200**	**0.045**
F2D10-C	**SNAP**	**1/7.16**	**3**	**2200**	**0.136**
F2D10	**SNAP**	**1/7.23**	**8**	**2012**	**0.398**
T22I11	InDel	1/7.37	10	620	1.613
F19G10	InDel	1/8.00	36	616	5.844
AthSO292	SSLP	1/10.86	23	148	15.541

Table 4.1: List of markers used for fine mapping of *spl7*, showing the type, location and recombination frequency (Rf) for each marker. Also shown are the number of recombinants and the total number of alleles. The markers used for the final stage of fine mapping are shown in bold.
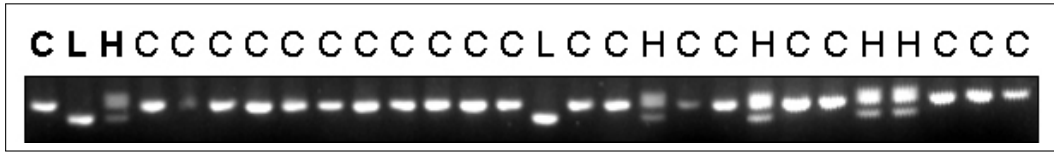
Figure 4.6: Extract from a fine mapping gel. Here the InDel marker T22I11 was used. Controls are on the left of the gel (shown in bold letters), the remaining samples are F$_2$ plants from the mapping population. C: Col-0 allele; L: *Ler* allele; H: heterozygous for Col-0 and *Ler*.
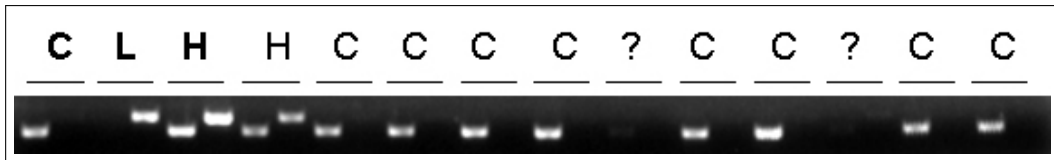
Figure 4.7: Extract from a fine mapping gel. Here the SNAP marker F2D10 was used. Controls are on the left of the gel (shown in bold letters), the remaining samples are F$_2$ plants from the mapping population. For SNAP markers, two PCRs are done for each sample; one specific for the Col allele, and one specific for the *Ler* allele. The Col band is on the left, the *Ler* band is on the right for each of the samples. C: Col-0 allele; L: *Ler* allele; H: heterozygous for Col-0 and *Ler*.

marker F19G10 and its four flanking markers F21B7, Nga63, SRP54A (to the left of F19G10), and AthSO392 (to the right of F9G10). Recombination freqencies for these five markers were calculated, and the rough location of *spl7* was found to be between the markers SRP54A and F19G10, in a region of approximately 2.75 Mb [Sorhagen, 2010]. Further mapping markers in this 2.75 Mb area were then utilised, and eventually the location of *spl7* was narrowed down to a region of approximately 230 kb between the markers T20H2-B and F2D10. 17 out of 1101 genotyped plants showed recombination events in this region. Additional mapping markers located in this 230 kb region were designed and the 17 recombinants were then used to further narrow down the

region of interest to a 60 kb area between the markers F5M15-B and F2D10-C [Table 4.2]. This area contained 19 gene loci from *At1g20480* to *At1g20640*.

Recombinants used for the final stage of fine mapping

	T20H2-B (1/7)	F14O10 (1/7.03)	F14O10-B (1/7.06)	F5M15-B (1/7.09)	F5M15 (1/7.11)	F2D10-B (1/7.13)	F2D10-C (1/7.16)	F2D10 (1/7.23)
# 12	C	C	C	C	C	C	H	H
# 25	C	C	C	C	C	C	C	H
# 96	C	C	C	C	C	C	H	H
# 673	C	C	C	C	C	C	C	H
# 887	H	H	H	C	C	C	C	C
# 932	H	H	C	C	C	C	C	C
# 1116	C	C	C	C	C	C	C	H
# 1261	H	H	C	C	C	C	C	C
# 1311	C	C	C	C	C	C	C	H
# 1515	H	H	H	H	H	C	C	C
# 1661	H	H	H	C	C	C	C	C
# 1689	H	H	H	H	C	C	C	C
# 2117	H	H	H	C	C	C	C	C
# 2129	H	H	H	H	C	C	C	C
# 2180	H	H	H	H	H	C	C	C
# 2188	C	C			C			H
# 2320	H	C	C	C	C	C	C	C
# Recom.	10	9	7	4	2	1	3	8
# Alleles	2010	2202	2200	2200	2202	2200	2200	2012
# Rf (%)	0.498	0.409	0.318	0.182	0.091	0.045	0.136	0.398

Table 4.2: Recombinants used for the final stage of fine mapping, showing the genotypes of the 17 recombinants used for fine mapping the 200 kb interval between markers T20H2-B and F2D10. C: homozygous Col-0, H: heterozygous for Col-0 and *Ler*.

4.4   *spl7* and *spl8* are mutations in *CAT3*

A 60 kb section on chromosome 1, located between markers F5M15-B and F2D10-C and containing 19 gene loci, was selected for sequencing [Fig. 4.8].

First, only the forward strand of *spl7* was sequenced and the sequences from the suppressor mutant were then compared to Col-0 sequences contained in the TAIR database [http://arabidopsis.org/] using the multiple alignment tool Kalign [http://www.ebi.ac.uk/Tools/msa/kalign/]. A G to A base change
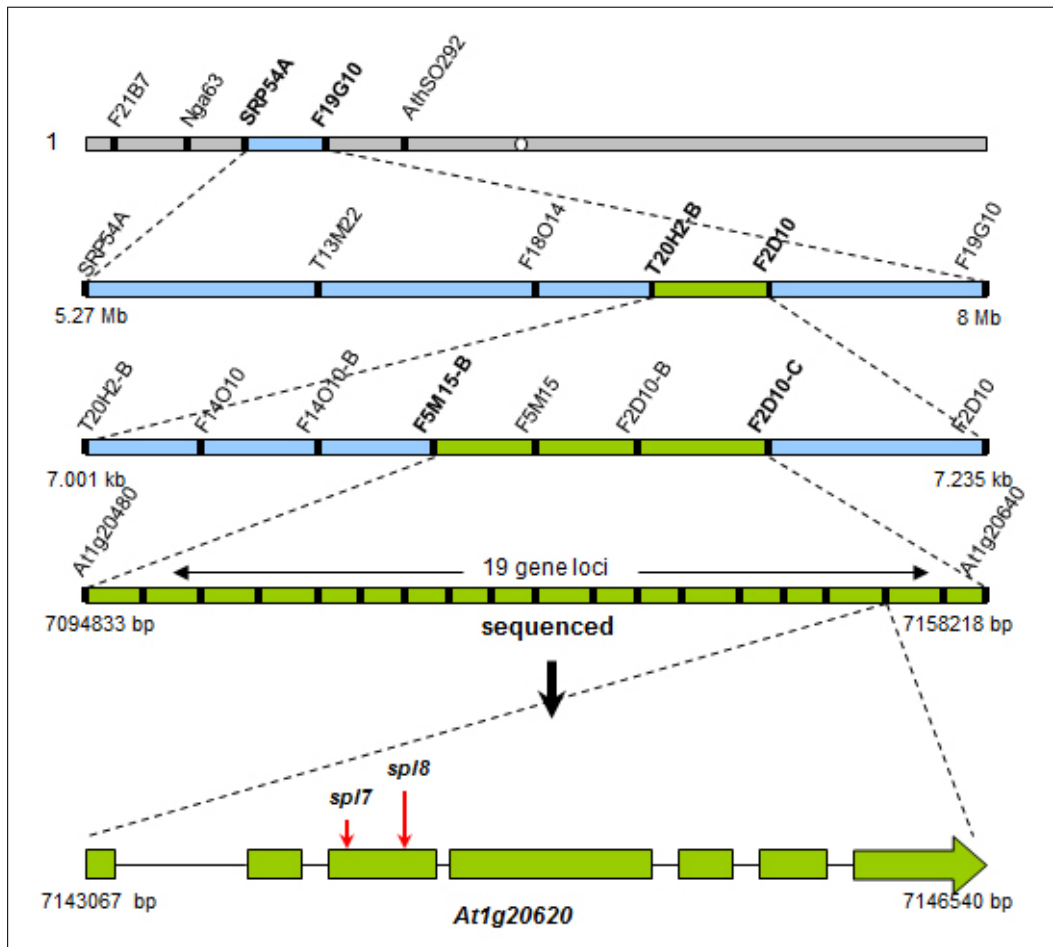
Figure 4.8: Schematic representation of the mapping procedure used to identify the *spl7* and *spl8* mutations. The mutations were found to be on chromosome 1 (shown in grey), between markers SRP54A and F19G10. This area (highlighted in blue) was then used for fine mapping of *spl7*. The location of the mutation was subsequently narrowed down to smaller and smaller regions (highlighted in green). Once the location of the mutation had been narrowed down to an area of 60 kb, all 19 genes in this region were sequenced. Mb, mega bases; kb, kilo bases; bp, base pairs. Adapted from [Sorhagen, 2010]

was identified in gene *At1g20620*. To confirm this was a real polymorphism, this gene was then sequenced in Col-0 and *atgsnor1-3*. To rule out sequencing artefacts, the reverse strand of *spl7* was also sequenced. The sequencing results confirmed that the observed base change in *At1g20620* was indeed a real mutation. Because *spl7* and *spl8* are allelic, only the gene *At1g20620* was sequenced in *atgsnor1-3 spl8* instead of the whole 60 kb section used for *atgsnor1-3 spl7*. As expected, the *spl8* mutation was also located in gene *At1g20620*, here a C to T mutation in a different location was observed.

```
ATGGATCCTTACAAGTATCGTCCTTCAAGCGCGTACAACGCCCCATTCTACACCACAAACGGTGGTGCTCCAGTCTCCAACAACATCTCTTCCC
TCACCATCGGAGAAAGAGGTCCGGTTCTTCTTGAGGATTATCATTTGATCGAGAAGGTTGCTAATTTCACCA[G/A]AGAGAGGATCCCTGAGA
GAGTGGTTCATGCTAGAGGAATCAGTGCTAAGGGTTTCTTTGAAGTCACCCATGACATTTCAAACCTCACTTGTGCTGATTTTCTCAGAGCCCC
TGGTGTTCAAACTCCGGTTATTGTCCGTTTCTCCACCGTTGTCCACGAACGTGCCAGTCCTGAAACCATGAGGGATATT[C/T]GTGGTTTTGC
TGTCAAGTTTTACACCAGAGAGGGAAACTTTGATCTTGTTGGGAACAACACTCCGGTGTTCTTCATCCGTGATGGGATTCAGTTCCCGGATGTT
GTCCACGCGTTGAAACCTAACCCGAAAACAAACATCCAAGAGTACTGGAGGATTCTGGACTACATGTCCCACTTGCCTGAGAGTTTGCTCACAT
GGTGCTGGATGTTTGATGATGTTGGTATTCCACAAGATTACAGGCACATGGAGGGTTTCGGTGTCCACACCTACACTCTTATTGCCAAATCTGG
AAAAGTTCTCTTTGTGAAGTTCCACTGGAAACCAACTTGTGGGATCAAGAATCTGACTGATGAAGAGGCCAAGGTTGTTGGAGGAGCCAATCAC
AGCCACGCCACTAAGGATCTCCACGATGCCATTGCATCTGGCAACTACCCCGAGTGGAAACTTTTCATCCAGACCATGGATCCTGCAGATGAGG
ATAAGTTTGACTTTGACCCACTTGATGTGACCAAGATCTGGCCTGAGGATATTTTGCCTCTGCAACCGGTTGGTCGCTTGGTTCTGAACAGGAC
CATTGACAATTTCTTCAATGAAACTGAGCAGCTTGCGTTCAACCCGGGTCTTGTGGTTCCTGGAATCTACTACTCAGACGACAAGCTGCTCCAG
TGTAGGATCTTTGCTTATGGTGACACTCAGAGACATCGCCTTGGACCGAATTATTTGCAGCTTCCAGTCAATGCTCCCAAATGTGCTCACCACA
ACAATCACCATGAAGGTTTTATGAACTTCATGCACAGAGATGAGGAGATCAATTACTACCCCTCAAAGTTTGATCCTGTCCGCTGCGCTGAGAA
AGTTCCCACCCCTACAAACTCCTACACTGGAATTCGAACAAAGTGCGTCATCAAGAAAGAGAACAACTTCAAACAGGCTGGAGACAGGTACAGA
TCATGGGCACCAGACAGGCAAGACAGGTTTGTTAAGAGATGGGTGGAGATTCTATCGGAGCCACGTCTCACCCACGAGATCCGCGGCATCTGGA
TCTCTTACTGGTCTCAGGCTGATCGATCCTTGGGACAGAAACTTGCAAGCCGTCTGAACGTGAGGCCAAGCATCTAG
```

Figure 4.9: The coding DNA sequence of *At1g20620* (*CAT3*). The bases that are mutated in *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are underlined and highlighted in yellow and red, respectively.

```
MDPYKYRPSSAYNAPFYTTNGGAPVSNNISSLTIGERGPVLLEDYHLIEKVANFT[R/K]ERIPERVVHARGISAKGFFEVTHDISNLTCADFL
RAPGVQTPVIVRFSTVVHERASPETMRDI[R/C]GFAVKFYTREGNFDLVGNNTPVFFIRDGIQFPDVVHALKPNPKTNIQEYWRILDYMSHLP
ESLLTWCWMFDDVGIPQDYRHMEGFGVHTYTLIAKSGKVLFVKFHWKPTCGIKNLTDEEAKVVGGANHSHATKDLHDAIASGNYPEWKLFIQTM
DPADEDKFDFDPLDVTKIWPEDILPLQPVGRLVLNRTIDNFFNETEQLAFNPGLVVPGIYYSDDKLLQCRIFAYGDTQRHRLGPNYLQLPVNAP
KCAHHNNHHEGFMNFMHRDEEINYYPSKFDPVRCAEKVPTPTNSYTGIRTKCVIKKENNFKQAGDRYRSWAPDRQDRFVKRWVEILSEPRLTHE
IRGIWISYWSQADRSLGQKLASRLNVRPSI
```

Figure 4.10: The protein sequence of *At1g20620* (*CAT3*). The amino acids that are changed as a result of the mutations in *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are underlined and highlighted in yellow and red, respectively. The *spl7* mutation leads to an arginine (R) to lysine (K) change, and *spl8* leads to an arginine (R) to cysteine (C) change.

At1g20620 encodes CAT3, which is one of the three catalases (CAT) in *Arabidopsis*. In the case of *atgsnor1-3 spl7* a G to A base change was found [Fig. 4.9], which resulted in an amino acid change from arginine to lysine in position 56 [Fig. 4.10]. In *atgsnor1-3 spl8* a C to T mutation was observed [Fig. 4.9], resulting in an amino acid change from arginine to cysteine in position 120 [Fig. 4.10].

4.5 *spl7* and *spl8* are located in conserved regions of CAT3

The three *Arabidopsis* catalases show a high sequence similarity [Fig. 4.11] and the *spl7* and *spl8* mutations are located in conserved regions. To determine if these regions are also conserved in different species, catalase protein sequences from *Arabidopsis*, human, mouse and maize were aligned to find conserved domains. Both *spl7* and *spl8* mutations were found to be in highly conserved regions [Fig. 4.12].

To further investigate the possible functional importance of the *spl7* and *spl8* mutations, a 3D model of *Arabidopsis* CAT3 was constructed [Fig. 4.13]. The crystal structure of *Arabidopsis* CAT3 is not known, so the 3D structure was modelled based on the catalase of *Exiguobacterium oxidotolerans* (UniProt accession number A2A136) [Hara et al., 2007] using the Phyre server [`http://www.sbg.bio.ic.ac.uk/phyre/`] [Kelley and Sternberg, 2009]. The resulting 3D model was then visualised using PyMol [Open-Source PyMOL 0.99rc6]. The active sites were identified using information available for human and

Figure 4.11: Alignment of catalase protein sequences from *Arabidopsis*. Grey areas with a star (*) below indicate identical amino acids; two dots (:) show conserved substitutions; one dot (.) indicates semi conserved amino acids (similar shape). The positions of the *spl7* and *spl8* mutations are highlighted in yellow and red, respectively. The active site histidine (H) and asparagine (N) are highlighted in blue.

Figure 4.12: Alignment of catalase protein sequences from different species: *Arabidopsis* CAT3, *Homo sapiens* catalase, *Mus musculus* catalase, and *Zea mays* CAT2. Grey areas with a star (*) below indicate identical amino acids; two dots (:) show conserved substitutions; one dot (.) indicates semi conserved amino acids (similar shape). The positions of the *spl7* and *spl8* mutations are highlighted in yellow and red, respectively. The active site histidine (H) and asparagine (N) are highlighted in blue.

Figure 4.13: 3D models of *Arabidopsis* CAT3.

(A) Ribbon diagram of CAT3, shown from three different angles. The *spl7* and *spl8* mutations are highlighted in yellow and red, respectively. The active site amino acids are shown in blue. Spirals represent α-helices and flat arrows represent β-sheets.

(B) Surface model of CAT3. The *spl7* mutation is highlighted in yellow, the *spl8* mutation is located within the protein and therefore not visible.

(C) Transparent surface model of CAT3. The *spl7* and *spl8* mutations are highlighted in yellow and red, respectively, and the active site amino acids in blue. Also shown are the heme axial ligand Tyr347 (dark grey), as well as Arg61 and Arg453 (light grey) which are also possibly involved in heme binding.

Arabidopsis catalases on the UniProt Protein KnowledgeBase website [`http://www.uniprot.org/`]. In human catalase the active site amino acids are His75 and Asn148, and Tyr358 is involved in heme binding as an axial ligand. These amino acids correspond to His64, Asn137, and Tyr347 in *Arabidopsis* CAT3. Three arginine residues, Arg72, Arg117, and Arg365, have been shown to be involved in heme binding in human catalase by forming salt bridges to the negatively charged heme carboxylate radical [Putnam et al., 2000]. Arg72 and Arg365 are conserved between human and *Arabidopsis* catalase and correspond to Arg61 and Arg354 in *Arabidopsis* CAT3, but the *Arabidopsis* equivalent of Arg117 could not be identified. The mutation *spl7* is located in the N-terminal region of CAT3, while the mutation *spl8* was found to be very close to the active site and also relatively close to the presumed heme binding site [Fig. 4.13].

4.6 The suppressor mutants show reduced catalase activity

Total leaf catalase activity of 7 week old Col-0, *atgsnor1-3*, *atgsnor1-3 spl7*, *atgsnor1-3 spl8*, *spl7*, and *spl8* plants was measured to test if the *spl7* and *spl8* mutations had an effect on CAT3 catalase activity [Fig. 4.14]. *cat1* [Hu et al., 2010], *cat2*, *cat3*, and *cat2 cat3* plants [Orendi et al., 2001] were included as controls. There was no significant difference in catalase activity of Col-0, *atgsnor1-3*, and *cat1*. *atgsnor1-3 spl7*, *spl7*, *spl8*, and *cat3* showed a reduction of total catalase activity of approximately 30-40%. *atgsnor1-3 spl8*

Figure 4.14: Total leaf catalase activity of 7 week old plants. Catalase activity is shown as μmol H_2O_2 min^{-1} mg total protein^{-1}. Error bars show SE (n=4; n=3 for *atgsnor1-3 spl8*). Values with different letters are significantly different (P<0.05, Duncan's MRT).
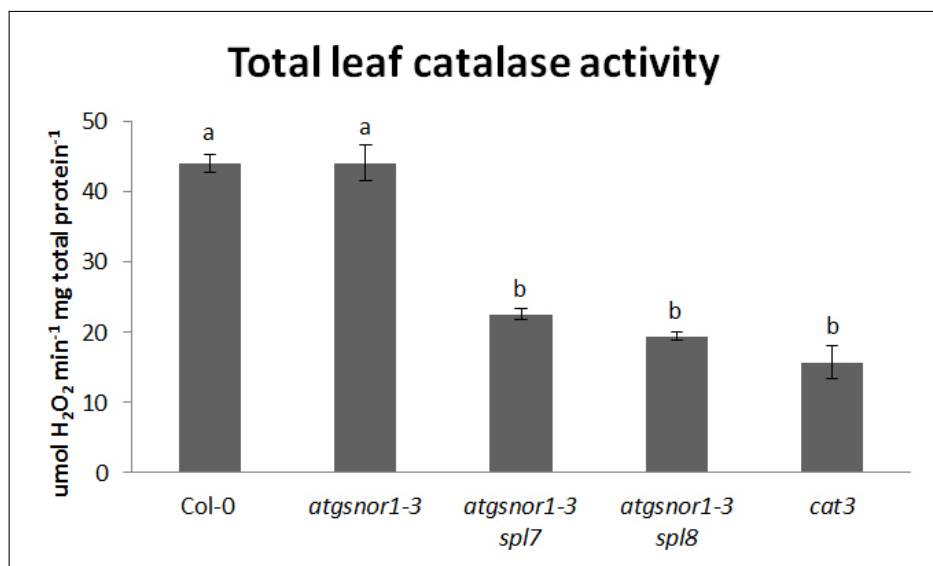
Figure 4.15: Total leaf catalase activity of 10 week old plants. Catalase activity is shown as μmol H_2O_2 min^{-1} mg total protein^{-1}. Error bars show SE (n=5; n=4 for *cat3*). Values with different letters are significantly different (P<0.01, Duncan's MRT).

only showed a reduction of catalase activitity of about 15% but this result is most likely due to experimental error. *cat2* and *cat2 cat3* plants showed around 70% and 90% reduction in catalase activity, respectively.

Measurements of catalase activity of Col-0, *atgsnor1-3*, *atgsnor1-3 spl7*, *atgsnor1-3 spl8*, and *cat3* were repeated, but with 10 week old plants [Fig. 4.15]. There was no significant difference between Col-0, and *atgsnor1-3*. *atgsnor1-3 spl7*, *atgsnor1-3 spl8*, and *cat3* showed a decrease of total catalase activity of approximately 50-65%.

4.7 Complementation of the suppressor mutations

To confirm that *spl7* and *spl8* were mutations in the *CAT3* gene, both *atgsnor1-3 spl7* and *atgsnor1-3 spl8* plants were transformed with a *2x35S::CAT3* construct. In the case of a successful complementation, it would be expected to observe an *atgsnor1-3* phenotype in transformants. Only one transformant in the *atgsnor1-3 spl8* background was obtained. When the T_2 plant were sprayed with BASTA, resistant and non-resistant plants segregated in a 3:1 ratio, indicating that the transformant most likely contained a single insert. The presence of the insert was also confirmed by PCR [Fig. 4.16].

The transformant lost apical dominance but unlike *atgsnor1-3* it did not show stunted growth [Fig. 4.17]. The *2x35S::CAT3* construct only partially complemented the mutation. However, transforming the suppressor mutants with either *pCAT3::CAT3* (*CAT3* driven by *CAT3* promoter) or

Figure 4.16: PCR to confirm presence of *2x35S::CAT3* insert in T$_2$ generation. From left to right: T$_2$ plants #1 to #9, negative control.
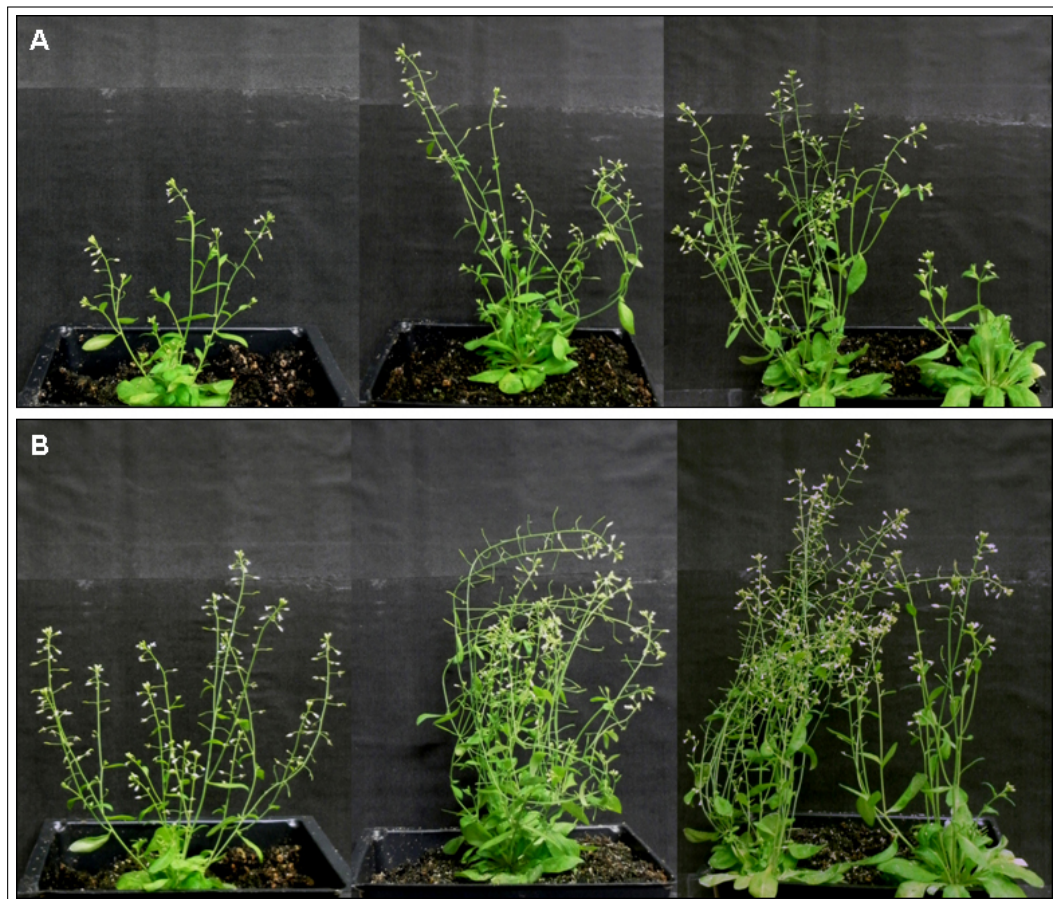
Figure 4.17: *2x35S::CAT3* construct in *atsgnor1-3 spl8* background. The plants shown here are the T$_2$ generation (from left to right: plant #4, #5, #6, #7). (A) 8 week old plants. Some of the plants showed a phenotype similar to *atgsnor1-3*, while others were slightly taller than *atgsnor1-3* (B) 9 week old plants. The plants were bushy but they were all taller than *atgsnor1-3* and reached about the same height as *atgsnor1-3 spl8*.

pCAT3::CAT2 (*CAT2* driven by *CAT3* promoter) fully complemented the mutation [Adil Hussain, unpublished results].

4.8 Discussion

The mutations *spl7* and *spl8*, which restore apical dominance in *atgsnor1-3* plants, were identified as mutations in *CAT3*, one of the three catalase genes in *Arabidopsis*. This is somewhat surprising, because CAT2 is the major catalase in *Arabidopsis* and accounts for approximately three quarters of total catalase activity, while CAT3 only accounts for about a quarter [Hu et al., 2010].

Both *spl7* and *spl8* mutations were found to be in regions that are highly conserved from plants to animals, suggesting that these areas probably play an important role in protein function and that any amino acid changes in these regions could have an impact on protein activity. Utilising a 3D model of CAT3 further confirmed that both mutations are in regions of the protein that are important for its functionality. The mutation *spl7* is located in the N-terminal part of the protein, which has been shown to be essential for tetramer formation [Ueda et al., 2003]. A mutation in this area could prevent the formation of tetramers and therefore render the protein inactive. The mutation *spl8* is located very close to the active site. Arginine, a basic amino acid, is changed to a thiol-containing amino acid, which could possibly alter the structure and/or the chemical properties of the active site, resulting either in reduced enzyme activity or in a complete loss of activity. The *spl8* mutation is also relatively close to the heme binding site, so it might be possible that it

interferes with heme binding.

In 10 weeks old plants, total leaf catalase activity of *atgsnor1-3 spl7*, *atgsnor1-3 spl8*, *spl7*, and *spl8* plants was not significantly different from total leaf catalase activity of *cat3* plants, indicating that both mutations probably result in a complete loss of CAT3 activity. Catalase activity was measured twice, and in the second experiment a much higher reduction of catalase activity was observed in *cat3* plants and the suppressor mutants. This is most likely due to the age of the plants; in the second experiment older plants were used. *CAT3* is upregulated with senescence while *CAT2* is downregulated [Zimmermann et al., 2006, Zentgraf, 2009], so in older plants CAT3 would account for a higher percentage of overall catalase activity. The observed values of catalase activity in the different *cat* mutants also match those reported in the literature [Hu et al., 2010, Du et al., 2008].

However, it cannot be ruled out that one or both of the mutations result in some residual CAT3 activity. To accurately test whether the *spl7* and *spl8* result in a complete loss of catalase activity, it would be necessary to recombinantly express the mutant proteins. It was attempted to recombinantly express catalase in *E. coli*, first using his-tagged protein and then using GST-tagged protein to increase solubility. Hemin was also added and *E. coli* was grown at a lower temperature, but it was not possible to obtain active catalase. A fairly recent publication described for the first time the expression and purification of a soluble and active plant catalase, rice catalase-A (cat-A) [Ray et al., 2012], showing that while it is possible to recombinantly express plant catalase in *E. coli*, it is certainly not a straightforward matter. Expression of cat-A as a Trx

fusion protein, overexpression of the chaperone Trigger Factor, and induction at low temperature were needed to obtain fully solubilised cat-A. Heme incorporation was achieved by adding δ-aminolevulinic acid (ALA), which is then synthesised to heme by *E. coli*. Replacing ALA with hemin did not result in active catalase.

The *spl7* and *spl8* mutations resulted in a 30-40% reduction of total catalase activity, about the same as in *cat3* plants. It could be speculated that this loss of catalase activity would result in higher H_2O_2 levels. However, previous studies only showed increased H_2O_2 levels in *cat2* mutants and not in *cat1* or *cat3* mutants [Du et al., 2008]. The different Arabidopsis catalases show tissue-specific expression patterns [Hu et al., 2010, Zimmermann et al., 2006, Du et al., 2008], so even if no difference in total H_2O_2 levels can be observed, it might be possible that there is a local increase in H_2O_2 levels in tissues where CAT3 is predominantly expressed, such as vascular tissues.

The *2x35S::CAT3* construct only partially complemented the mutation, which could be due to several different reasons. A likely explanation is that the constitutive *35S* promoter has a higher expression level than the *CAT3* promoter, which would result in higher catalase activity in the transformant compared to *atgsnor1-3*. Furthermore, the two promoters might be expressed in different tissues. The location where the *2x35S::CAT3* construct was inserted in the genome might also play a role. Using the *CAT3* promoter complementated the mutation, confirming that the *spl7* and *spl8* mutations are indeed in *CAT3*. Furthermore, *CAT2* driven by the *CAT3* promoter also complemented the mutation, while CAT3 driven by the *35S* promoter only

partially complemented, which shows that both the expression levels as well as the location of *CAT3* expression play a very important role. Furthermore, the *2x35S::CAT3* plants uncouple apical dominance from growth defects, and could therefore provide a useful tool to further study the role of *atgsnor1-3* in different phenotypes.

A NO accumulation mutant in rice, *nitric oxide excess 1* (*noe1*), provides a further link between GNSOR1 and catalase [Lin et al., 2012]. *noe1* encodes a rice catalase, *OsCATC*, which corresponds to *Arabidopsis* CAT2 [Mhamdi et al., 2010]. *noe1* plants show increased H_2O_2 levels in leaves, which leads to activation of nitrate reductase and an increase in NO levels. When exposed to high light or high temperatures, *noe1* plants develop PCD. Overexpression of GNSOR1 leads to a reduction of NO levels in *noe1* plants, and also alleviates cell death. *noe1* and the suppressor mutants cannot be compared directly because different classes of catalase are involved, but nevertheless *noe1* show a connection between GSNOR1 and catalase, and between H_2O_2 and NO levels, and also provide further proof of their involvement in cell death. It also worth noting that H_2O_2 and NO induction are dependent on light levels and temperature, and this needs to be taken into consideration when sampling. It might also be possible to use light- or temperature-induced cell death as an alterative screening method.

Chapter 5

Characterisation of *atgsnor1-3* suppressor mutants

5.1   GSNOR1

GSNOR1 plays a crucial role in regulating plant defence responses. In *Arabidopsis*, several T-DNA insertion lines have been characterised which show either an increase (*atgsnor1-1* and *atgsnor1-2*) or a loss (*atgsnor1-3*) of GSNOR1 activity [Feechan et al., 2005]. Basal SNO levels in *atgsnor1-3* are increased compared to wild type plants, and increase even further after pathogen challenge, while *atgsnor1-1* and *atgsnor1-2* show reduced SNO levels. *atgsnor1-3* plants are compromised in R-gene mediated resistance, and both CC-NB-LRR and TIR-NB-LRR pathways are affected. Basal resistance against virulent bacteria and oomycetes is also compromised, as well as non-host resistance. Basal and pathogen-induced SA levels are very low in *atgsnor1-3*. *PR1* ex-

pression, which marks the expression of SA-dependent genes, is reduced and delayed in *atgsnor1-3*, and increased in *atgsnor1-1*. Exogenous SA application results in reduced and delayed *PR1* expression in *atgsnor1-3* plants, indicating that they are impaired in both SA synthesis and SA signalling. *atgsnor1-3* plants also accumulate less ROS after pathogen challenge and show increased HR [Yun et al., 2011]. While cell death alone is not sufficient to limit bacterial infection, it plays an important role in resistance against oomycetes. When challenged with an avirulent oomycete, *atgsnor1-3* plants are as resistant as wild type plants [Yun et al., 2011]. GSNOR1 also plays a role in thermotolerance; *atgsnor1-3* plants are heat-sensitive [Lee et al., 2008]. Furthermore, *atgsnor1-3* plants are resistant to paraquat, which induces the production of superoxide and hydrogen peroxide [Chen et al., 2009]. Paraquat resistance might be due to increased NO levels in *atgsnor1-3* plants; it has been speculated that NO might react with superoxide to form the less toxic peroxynitrite anion.

Contradictory results were obtained using *GSNOR1* overexpresser and antisense lines [Rustérucci et al., 2007]. The antisense lines exhibited enhanced basal resistance to oomycetes and were not impaired in *R*-gene mediated resistance. They also showed enhanced SAR and constitutive *PR1* expression, which was induced to even higher levels after infection with virulent pathogens. The overexpresser lines showed reduced SAR. SA levels and HR were not different from wild type in both antisense and overexpresser lines. Exogenous SA application induced strong and fast *PR1* induction in the antisense lines, while the overexpresser lines failed to induce *PR1* expression systemically [Espunya

et al., 2012]. Antisense *GSNOR1* plants also showed higher levels of PDF1/2, which is involved in JA-dependent wound responses [Espunya et al., 2012].

While these opposing results might seem surprising at first, it is worth noting that one group used T-DNA lines [Feechan et al., 2005, Yun et al., 2011], while the other group used antisense and overexpresser lines [Rustérucci et al., 2007, Espunya et al., 2012]. SNO levels increase after pathogen infection and once a certain threshold of S-nitrosylation is reached, SA synthesis, SABP3 function and NPR1 monomerisation are blocked, which negatively regulates the plant defence response [Malik et al., 2011, Yu et al., 2012]. While the T-DNA lines show a complete loss of GNSOR1 function, it is quite possible that the antisense lines exhibit residual GNSOR1 activity and therefore have SNO levels that are below the threshold required to negatively regulate the plant immune response.

5.2 Disease resistance

atgsnor1-3 plants are compromised in basal, *R*-gene mediated, and non-host resistance [Feechan et al., 2005]. To test whether the suppressor mutants have recovered wild-type basal and *R*-gene mediated resistance, *atgsnor1-3 spl7* and *atgsnor1-3 spl8* plants were inoculated with virulent *Pst* DC3000, and with several avirulent *Pst* DC3000 strains. *spl7* and *spl8* plants were also included to test whether the *spl7* and *spl8* mutations on their own have an effect on disease resistance.

5.2.1 *atsgnor1-3 spl7* and *atsgnor1-3 spl8* are susceptible to *Pst* DC3000

atgsnor1-3 spl7 and *atgsnor1-3 spl8* plants were inoculated with *Pst* DC3000 and colony forming units (CFU) per cm^2 leaf were counted 0 and 5 days post inoculation (dpi) [Fig. 5.1]. There was no significant difference between *atgsnor1-3* and the supressor mutants. *atgsnor1-3 spl7* and *atgsnor1-3 spl8* have not recovered wild type basal disease resistance and are still as susceptible as *atgsnor1-3* to virulent pathogens. These results matched previous experiments, which also showed that the suppressor mutants have not recovered wild type basal resistance [Sorhagen, 2010]. *spl7* and *spl8* plants showed wild type basal resistance, indicating that the *spl7* and *spl8* mutations on their own do
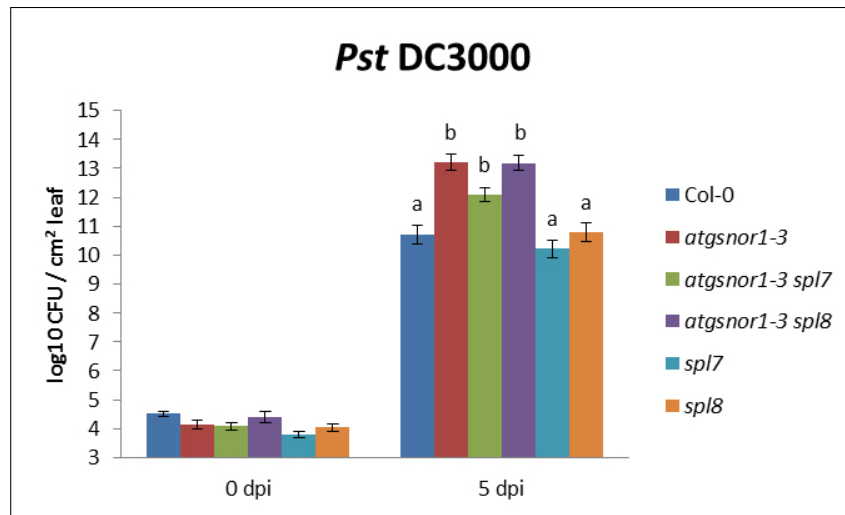
Figure 5.1: Basal disease resistance of suppressor mutants. 4 week old plants were inoculated with 10^5 colony forming units (CFU) ml^{-1} virulent *Pst* DC3000 and CFU per cm^2 leaf were counted 0 and 5 days post inoculation (dpi). Error bars show SE (n=7-16 for 0 dpi; n=14-16 for 5 dpi). Values with different letters are significantly different (P<0.01, Duncan's MRT).

not have an impact on disease resistance to virulent pathogens.

5.2.2 *atsgnor1-3 spl7* and *atsgnor1-3 spl8* are partially resistant to avirulent *Pst* DC3000

In order to test *R*-gene mediated resistance, *atgsnor1-3 spl7* and *atgsnor1-3 spl8* plants were inoculated with avirulent *Pst* DC3000 (*avrB*), (*avrRpm1*), and (*avrRps4*). *R*-gene mediated resistance to *Pst* DC3000 (*avrB*) and (*avrRpm1*) is dependent on NDR1, while resistance to *Pst* DC3000 (*avrRps4*) requires EDS1/PAD4. The effectors AvrB and AvrRpm1 are recognised through the CC-NB-LRR protein RPM1 [Grant et al., 1995], while AvrRps4 is recognised through the TIR-NB-LRR protein RPS4 [Gassmann et al., 1999].

atgsnor1-3 spl7 showed intermediate resistance to *Pst* DC3000 (*avrRps4*), and *atgsnor1-3 spl8* exhibited intermediate to wild type resistance [Fig. 5.2]. These results matched the disease symptoms after inoculation with *Pst* DC3000 (*avrRps4*); *atgsnor1-3* showed pronounced yellowing of the leaves, while disease symptoms in the suppressor mutants were less strong than in *atgsnor1-3* but more severe than in Col-0 [Fig. 5.3]. Both suppressor mutants showed intermediate disease resistance to *Pst* DC3000 (*avrB*) and to *Pst* DC3000 (*avrRpm1*), while *spl7* and *spl8* were not significantly different from wild type plants [Figs. 5.4 and 5.5].

It can be concluded that the suppressor mutants have partially recovered wild type *R*-gene mediated resistance. *atgsnor1-3 spl8* plants appear to be slightly more resistant than *atgsnor1-3 spl7* plants and possibly show wild

type resistance to *Pst* DC3000 (*avrRps4*) but the results are not robust enough
to say this with certainty.
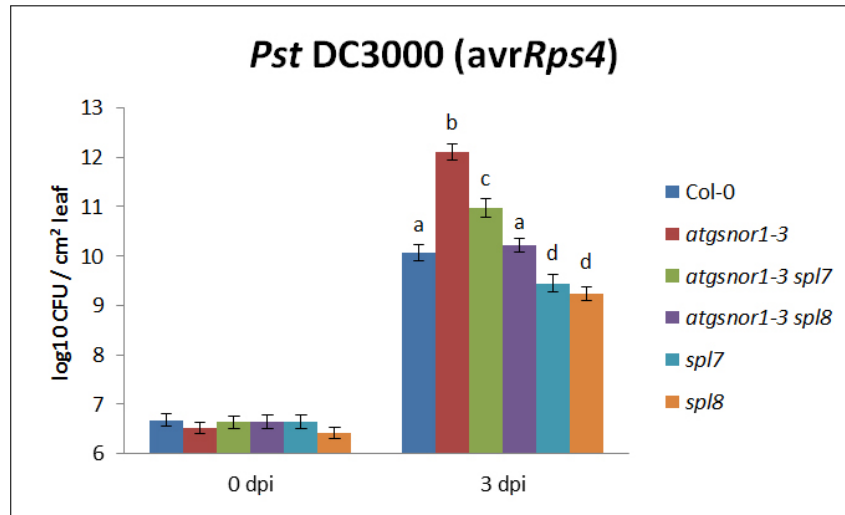
Figure 5.2: *R*-gene mediated resistance (*avrRps4*) of suppressor mutants. 4
week old plants were inoculated with 10^6 colony forming units (CFU) ml^{-1}
avirulent *Pst* DC3000 (*avrRps4*) and CFU per cm^2 leaf were counted 0 and 3
days post inoculation (dpi). Error bars show SE (n=16). Values with different
letters are significantly different (P<0.01, Duncan's MRT). Results of one
representative experiment are shown, the experiment was repeated six times
with similar results, and *atgsnor1-3 spl8* showed wild type to intermediate
disease resistance.

Figure 5.3: *Arabidopsis* leaves 3 days after inoculation with 10^6 CFU ml^{-1}
Pst DC3000 (*avrRps4*). From left to right: Col-0, *atgsnor1-3*, *atgsnor1-3 spl7*,
atgsnor1-3 spl8, *spl7*, *spl8*.
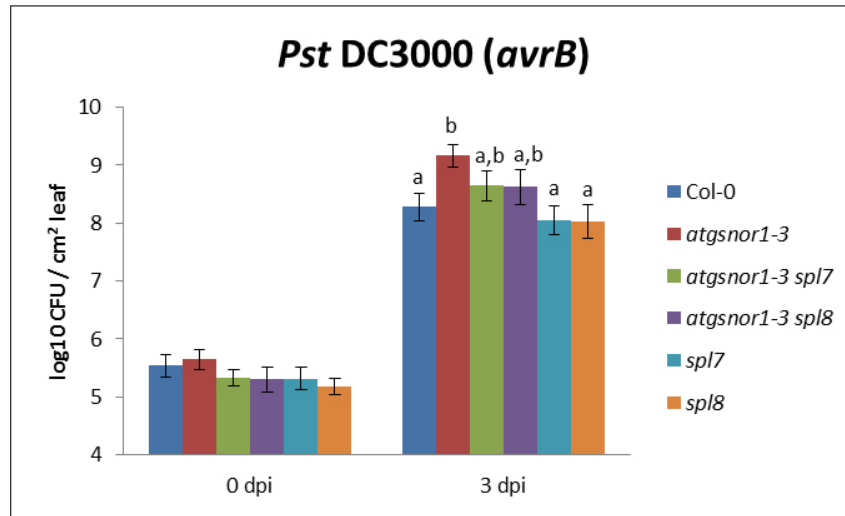
Figure 5.4: *R*-gene mediated resistance (*avrB*) of suppressor mutants. 4 week old plants were inoculated with 10^6 CFU ml^{-1} avirulent *Pst* DC3000 (*avrB*) and CFU per cm^2 leaf were counted 0 and 3 days post inoculation (dpi). Error bars show SE (n=16). Values with different letters are significantly different (P<0.05, Duncan's MRT). Results of one representative experiment are shown, the experiment was repeated three times with similar results.
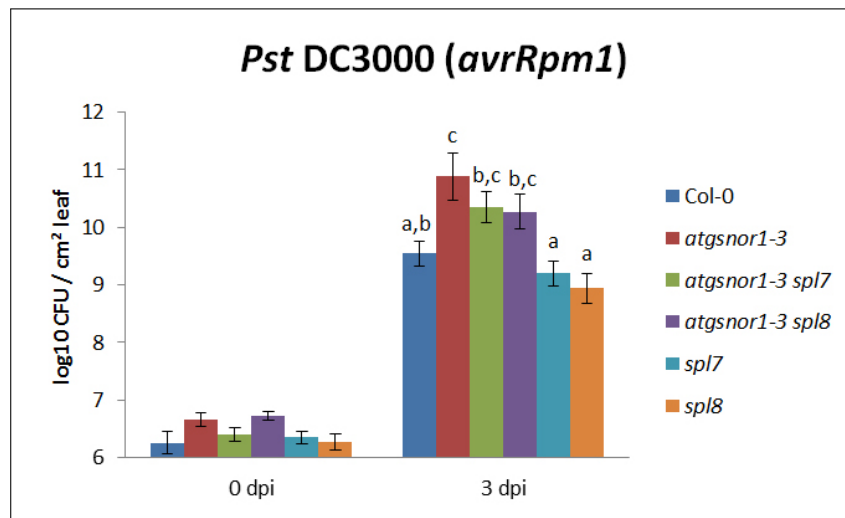
Figure 5.5: *R*-gene mediated resistance (*avrRpm1*) of suppressor mutants. 4 week old plants were inoculated with 10^6 CFU ml^{-1} avirulent *Pst* DC3000 (*avrRpm1*) and CFU per cm^2 leaf were counted 0 and 3 days post inoculation (dpi). Error bars show SE (n=16). Values with different letters are significantly different (P<0.05, Duncan's MRT). Results of one representative experiment are shown, the experiment was repeated seven times with similar results.

5.2.3 *atsgnor1-3 spl7* and *atsgnor1-3 spl8* might be partially resistant to *Psp*

The suppressor mutants were inoculated with the non-host pathogen *Pseudomonas syringae* pv *phaseolicola* (*Psp*) and colony forming units (CFU) per cm^2 leaf were counted 0 and 5 days post inoculation (dpi) [Fig. 5.6]. *atsgnor1-3 spl7* and *atsgnor1-3 spl8* showed intermediate resistance compared to Col-0 and *atgsnor1-3*, and there was no significant difference between the suppressor mutants and either Col-0 or *atgsnor1-3*. *spl7* and *spl8* showed wild type resistance. This experiment was repeated several times but in all subsequent experiments there was no significant difference between Col-0 and *atgsnor1-3*.
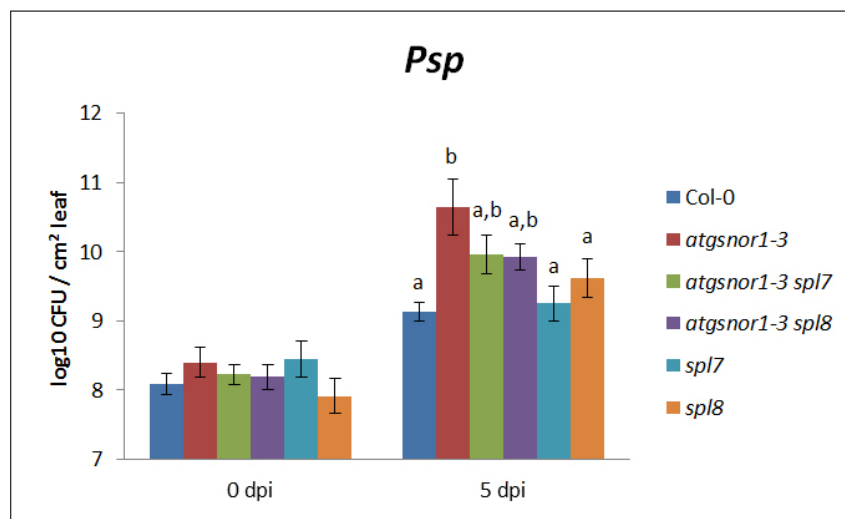
Figure 5.6: Non-host resistance of suppressor mutants to *Psp*. 4 week old plants were inoculated with 10^7 colony forming units (CFU) ml^{-1} *Psp* and CFU per cm^2 leaf were counted 0 and 5 days post inoculation (dpi). Error bars show SE (n=8). Values with different letters are significantly different (P<0.05, Duncan's MRT).

5.3 Hypersensitive response

atgsnor1-3 plants show increased HR after pathogen challenge [Yun et al., 2011]. To test whether the suppressor mutants had recovered wild type HR, they were challenged with different avirulent *Pst* DC3000 strains and leaves were stained with Trypan Blue 24 hours post inoculation (hpi) to visualise cell death. Electrolyte leakage was also used to quantify hypersensitive cell death.

Both suppressor mutants showed wild type HR in response to *Pst* DC3000 (*avrB*) and to *Pst* DC3000 (*avrRpm1*), while HR in *atgsnor1-3* plants was increased in response to both pathogens [Figs. 5.7 and 5.8]. Both suppres-
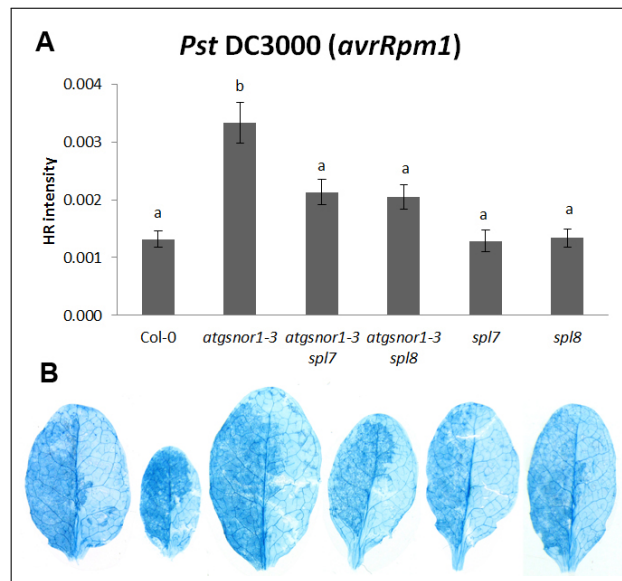
Figure 5.7: (A) HR intensity 24 hours post inoculation (hpi) with 10^7 CFU ml^{-1} *Pst* DC3000 (*Rpm1*). Error bars show SE (n=10-12). Values with different letters are significantly different (P<0.01, Duncan's MRT); for P<0.05, *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are significantly different from the other four plant lines. (B) Trypan Blue stained *Arabidopsis* leaves 24 hours after inoculation with 10^7 CFU ml^{-1} *Pst* DC3000 (*Rpm1*). The left half of each leaf was inoculated, the right half was left untreated. From left to right: Col-0, *atgsnor1-3, atgsnor1-3 spl7, atgsnor1-3 spl8, spl7, spl8*.
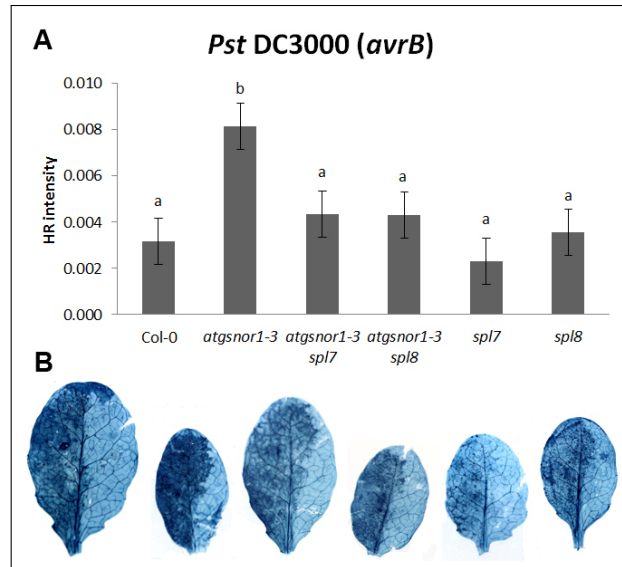
Figure 5.8: (A) HR intensity 24 hours post inoculation (hpi) with 10^7 CFU ml^{-1} *Pst* DC3000 (*avrB*). Error bars show SE (n=12). Values with different letters are significantly different (P<0.01, Duncan's MRT). Results of one representative experiment are shown, the experiment was repeated three times with similar results. (B) Trypan Blue stained *Arabidopsis* leaves 24 hours after inoculation with 10^7 CFU ml^{-1} *Pst* DC3000 (*avrB*). The left half of each leaf was inoculated, the right half was left untreated. From left to right: Col-0, *atgsnor1-3*, *atgsnor1-3 spl7*, *atgsnor1-3 spl8*, *spl7*, *spl8*.
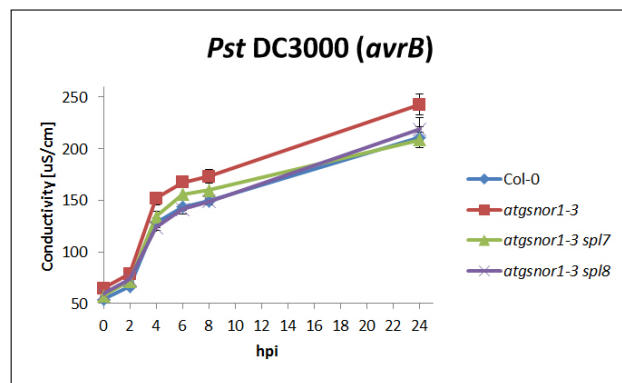
Figure 5.9: Electrolyte leakage after inoculation with *Pst* DC3000 (*avrB*). Error bars show SE (n=4). Col-0 and *atgsnor1-3* are significantly different; at 4 hpi *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are significantly different from Col-0 and *atgsnor1-3*, at the other time points *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are not significantly different from either Col-0 or *atgsnor1-3* (P<0.05, Duncan's MRT).
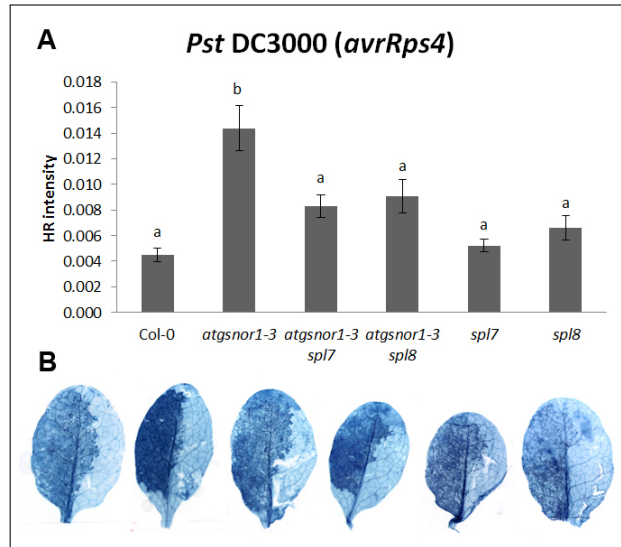
Figure 5.10: (A) HR intensity, 24hpi, 10^7 CFU ml^{-1} *Pst* DC3000 (*avrRps4*). Error bars show SE (n=8-12). Values with different letters are significantly different (P<0.01, Duncan's MRT); for P<0.05, *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are significantly different from the other four plant lines. Results of one representative experiment are shown, the experiment was repeated twice with similar results. (B) Trypan Blue stained *Arabidopsis* leaves 24 hours after inoculation with 10^7 CFU ml^{-1} *Pst* DC3000 (*avrRps4*). The left half of each leaf was inoculated, the right half was left untreated. From left to right: Col-0, *atgsnor1-3*, *atgsnor1-3 spl7*, *atgsnor1-3 spl8*, *spl7*, *spl8*.
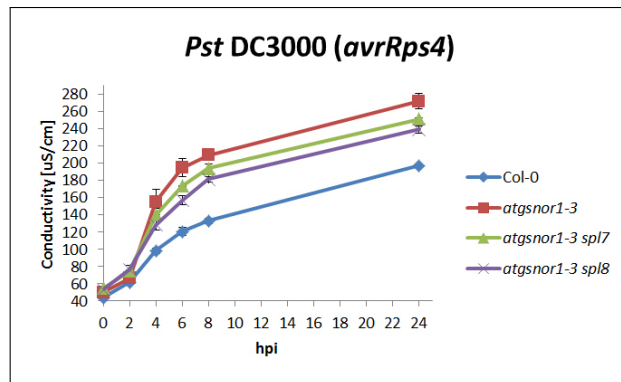
Figure 5.11: Electrolyte leakage after inoculation with 10^8 CFU ml^{-1} *Pst* DC3000 (*Rps4*). Error bars show SE (n=4). Col-0 and *atgsnor1-3* are significantly different; up to 4 hpi *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are significantly different from Col-0 but not from *atgsnor1-3*, at 6 hpi and later *atgsnor1-3 spl7* and *atgsnor1-3 spl8* are significantly different from both Col-0 and *atgsnor1-3* (P<0.05, Duncan's MRT).

sor mutants also exhibited wild type HR after challenge with *Pst* DC3000 (*avrRps4*), while *atgsnor1-3* showed increased HR [Fig. 5.10]. Measuring electrolyte leakage after inoculation with *Pst* DC3000 (*avrB*) or *Pst* DC3000 (*avrRps4*) corroborated these results to an extent; both suppressor mutants showed less electrolyte leakage than *atgsnor1-3* but higher electrolyte leakage than Col-0 [Figs. 5.9 and 5.11]. However, variation between different electrolyte leakage measurements was substantial, so these results would appear less reliable than results from Trypan Blue Staining, which was repeated several times with very similar results.

5.4 Salicylic acid levels and *PR1* expression

After pathogen infection, a rise in salicylic acid (SA) levels can be observed in wild type plants but not in *atgsnor1-3* plants [Feechan et al., 2005]. SA levels in the suppressor mutants were measured before and after challenge with *Pst* DC3000 (96 hpi) and *Pst* DC3000 (*Rps4*) (48 hpi). Both basal SA levels and SA levels after pathogen infection were very low in the suppressor mutants, just like in *atgsnor1-3* [Sorhagen, 2010].
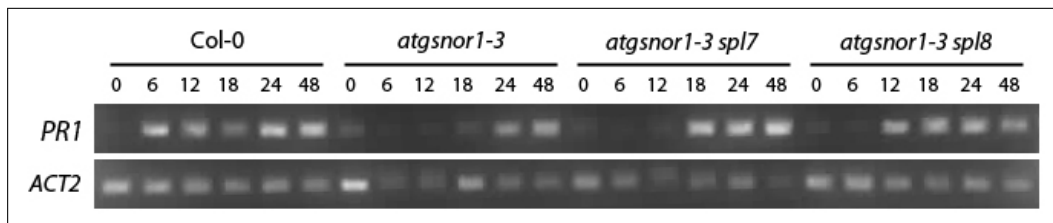
Figure 5.12: *PR1* expression in Col-0, *atgsnor1-3*, *atgsnor1-3 spl7*, and *atgsnor1-3 spl8*; 0, 6, 12, 18, 24, and 48 h after inoculation with *Pst* DC3000 (*Rps4*). *Actin2* (*ACT2*) used as control.

atgsnor1-3 plants are also compromised in *PR1* expression; in response to pathogen infection or SA treatment they show reduced and delayed *PR1* expression [Feechan et al., 2005]. After inoculation with *Pst* DC3000 (*Rps4*), the suppressor mutants showed delayed *PR1* expression compared to Col-0 but seemed to show slightly faster *PR1* expression than *atgsnor1-3* [Fig. 5.12]. However, this experiment was only done once, so these results should probably be treated with caution.

5.5 Sensitivity to ROS and GSNO

Col-0, *atgsnor1-3*, *atgsnor1-3 spl7* and *atgsnor1-3 spl8* seeds were sown on 1/2 MS plates containing NO or ROS donors and cotyledon development frequency after 8 days was used to determine their sensitivity to GNSO and ROS [Fig. 5.13] [Sorhagen, 2010]. *atgsnor1-3* was sensitive to the NO donor GSNO and to H_2O_2 compared to wild type. Wild type plants were susceptible to the superoxide donor methyl viologen (MV), whereas *atgsnor1-3* was resistant. Both suppressor mutants were extremely susceptible to GNSO, and did not germinate at all. Both *atgsnor1-3* and the suppressor mutants did not germinate at all on H_2O_2 plates, while almost all Col-0 seeds germinated. *atgsnor1-3 spl7* was still resistant to MV, whereas *atgsnor1-3 spl8* had recovered some sensitivity to MV, but was not anywhere near as sensitive as Col-0.

To test whether loss of GNSOR1 function or loss of catalase function is predominantly responsible for the suppressor mutants sensitivity to H_2O_2, various *cat* mutants as well as *atgsnor1-3 cat* double and triple mutants were
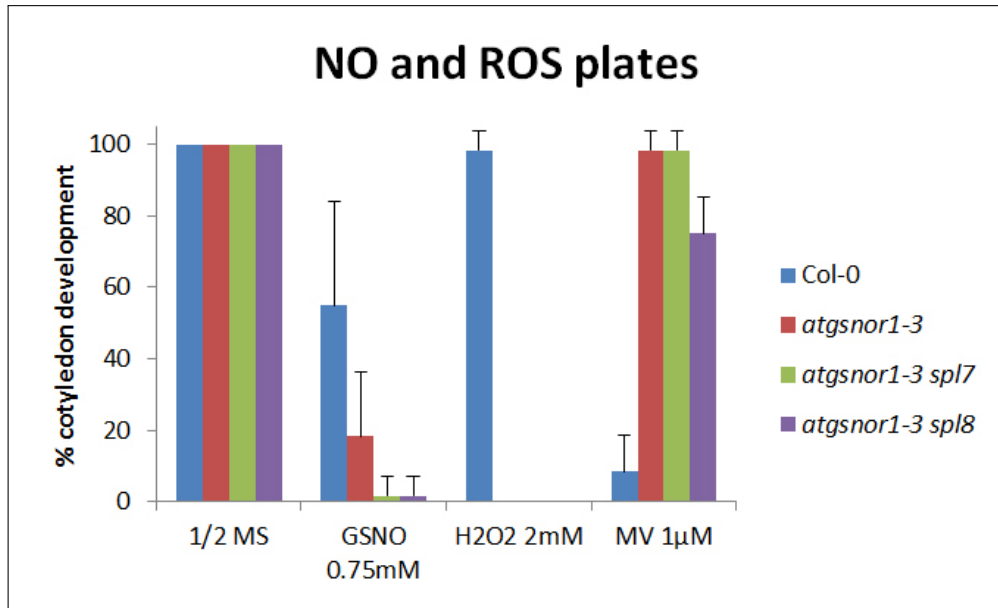
Figure 5.13: Sensitivity to ROS and GSNO. Cotyledon development frequence was observed after 8 days. Error bars show 95% confidence intervals. [Data obtained by Kirsti Sorhagen]
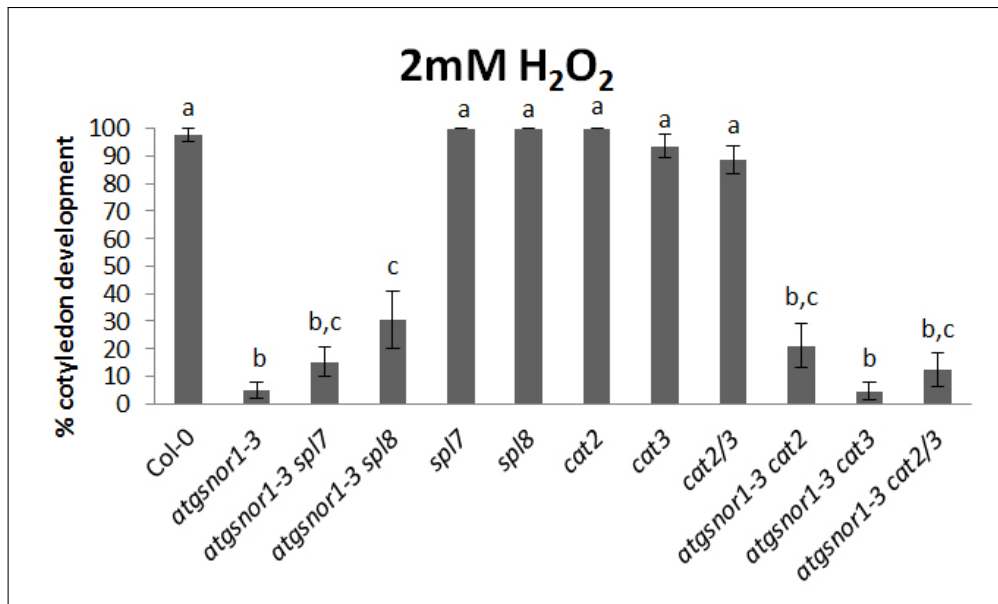
Figure 5.14: Sensitivity to H_2O_2. Cotyledon development frequency was observed after 10 days. Error bars show SE (n=5; n=4 for *atgsnor1-3 cat2*). Values with different letters are significantly different (P<0.01, Duncan's MRT).

grown on H_2O_2 plates [Fig. 5.14]. There was no significant difference between *spl7*, *spl8*, *cat2*, *cat3*, *cat2 cat3*, and wild type plants. All these lines showed almost 100% cotyledon development. *atgsnor1-3*, *atgsnor1-3 spl7*, *atgsnor1-3 spl8*, *atgsnor1-3 cat2*, *atgsnor1-3 cat3*, and *atgsnor1-3 cat2 cat3*, on the other hand, showed drastically reduced cotyledon development frequency between approximately 5 to 30 %. All these lines were significantly different from wild type. These results show that even almost complete loss of catalase activity, as in *cat2 cat3*, does not affect growth on H_2O_2, whereas loss of GSNOR1 function, either without or in combination with loss of catalase function, does have an effect on tolerance to H_2O_2.

5.6 Discussion

The suppressor mutants are still susceptible to virulent *Pst* DC3000 but both *atgsnor1-3 spl7* and *atgsnor1-3 spl8* have partially recovered disease resistance to avirulent *Pst* DC3000. In a few experiments it was observed that *atgsnor1-3 spl8* is more resistant than *atgsnor1-3 spl7* but whether these results are reliable and not just due to experimental error remains to be seen. SA levels in the suppressor mutants are still low, so it can be ruled out that SA-mediated signalling plays a role in partially restoring disease resistance. However, SA-independent signalling in response to avirulent pathogens has been reported, so this could be a possible explanation for the partial recovery of disease resistance to avirulent *Pst* DC3000. *SA induction-deficient* (*sid*) mutants do not accumulate SA after pathogen inoculation. They are more susceptible to *Pst* DC3000 and *Hyaloperonospora arabidopsidis* compared to wild type and show reduced

PR1 expression, but *PR2* and *PR5* are expressed normally and camalexin is also accumulated normally in response to pathogen infection [Nawrath and Metraux, 1999]. *ndr1* loss-of-function plants are susceptible to *Pst* DC3000 (*avrRpt2*) but they still have *PR1* expression and SAR, and can mount HR in response to *Pst* DC3000 (*avrB*) [Shapiro and Zhang, 2001]. These results show that there are two pathways acting together to induce *PR* gene expression and SAR. One pathway is NPR1-independent and involves signals associated with hypersensitive cell death, while the other one involves SA accumulation and is NPR1-dependent [Zhang and Shapiro, 2002]. Some *RPP* (*resistance to Psp*) genes also function in a SA-independent manner [McDowell et al., 2000]. RPP4 requires EDS1 and SA, and is partially dependent on NDR1 and NPR1; RPP2 however only requires EDS1 but not NPR1 or SA. RPP7 and probably RPP8 are also independent of SA and NPR1, and partially dependent on additive functions of EDS1 and NDR1. *nahG* plants, which cannot accumulate SA, and *ndr1* plants are both compromised in RPS2-mediated responses, but not in RPM1-mediated responses [Tao et al., 2003]. EDS1 appears to be of particular interest because it has been shown to transduce ROS-derived signals in biotic and abiotic stress signalling [Rustérucci et al., 2001, Mateo et al., 2004, Mühlenbock et al., 2008, Straus et al., 2010] and also plays a role in SA-independent signalling [Bartsch et al., 2006]. The partial restoration of disease resistance in the suppressor mutants might be due to the activcation of ROS-dependent, but SA-independent defence pathways.

The suppressor mutants seem to have partially recovered wild type *PR1* expression after infection with *Pst* DC3000 (*avrRps4*). However, before draw-

ing any conclusions from these results, it would be necessary to repeat this experiment and also to determine *PR1* expression after challenge with other pathogens and after SA treatment. It would also be useful to look at the expression of other genes involved in either SA-dependent or SA-independent defence responses.

The suppressor mutants show wild type HR in response to inoculation with avirulent *Pst* DC3000, while *atgsnor1-3* plants show accelerated cell death. However, cell death alone is not sufficient to stop a bacterial infection, and HR can be uncoupled from disease resistance. *ndr1* plants show RPM1-dependent HR after infection with various avirulent pathogens, but this is not sufficient to stop the bacterial spread [Century et al., 1995]. The balance of ROS and NO is important for regulating HR and it has been suggested that fine tuned concentrations of H_2O_2 and NO are important for initiating cell death [Delledonne et al., 2001]. Increased H_2O_2 levels in the suppressor mutants, caused by loss of CAT3 function, could restore the balance of ROS and NO, leading to wild type HR in the suppressor mutants. However, only loss of CAT2 function has been reported to lead to an increase in total H_2O_2 levels [Du et al., 2008], so increased H_2O_2 levels might only be observed in certain tissues in which CAT3 is predominantly expressed. It would be useful to measure total H_2O_2 levels in the suppressor mutants, and also to determine if H_2O_2 concentration is increased only in certain tissues. It was repeatedly attempted to use diaminobenzidine (DAB) staining to determine the intensity and location of H_2O_2 production in the suppressor mutants compared to wild type and *atgnsor1-3* plants but so far without success, so optimisation of the

staining procedure might be necessary. It is also worth noting that quantifying H_2O_2 content is notoriously difficult and there are huge discrepancies between H_2O_2 values reported in the literature [Queval et al., 2008].

Both suppressor mutants are still susceptible to GNSO and H_2O_2, and are resistant to the superoxide donor MV, but *atgsnor1-3 spl8* has lost some resistance to MV compared to *atgsnor1-3* and *atgsnor1-3 spl7*. So even though SNO levels have not been measured, these results indicate that SNO levels are most likely still high in the suppressor mutants and that a reduction or loss of CAT3 function does not influence SNO levels. *spl7* and *spl8* plants, as well as *cat2*, *cat3*, and *cat2 cat3* are all resistant to H_2O_2, while *atgsnor1-3 spl7* and *atgsnor1-3 spl8*, as well as *atgsnor1-3 cat2*, *atgsnor1-3 cat3*, and *atgsnor1-3 cat2 cat3* all show impaired cotyledon development when exposed to H_2O_2, just like *atgsnor1-3*. From these results it can be concluded that the suppressor mutants' sensitivity to H_2O_2 is due to loss of GSNOR1 function and not influenced by loss of catalase activity. To further disentangle thecontributions of H_2O_2 and NO to the phenotype of the suppressor mutants, it could be useful to treat their leaves with CAT (an H_2O_2 scavenger), sodium nitroprusside (an NO donor) and/or PTIO (depletes NO), as previously described [Lin et al., 2012].

Chapter 6

Investigating the roles of other redox-related genes

6.1 Catalases

Catalases are tetrameric heme-containing enzymes which are present in all aerobic organisms. They can act over a wide range of H_2O_2 concentrations and provide the cell with an energy efficient mechanism to degrade H_2O_2 because they do not require any reductants [Zentgraf, 2009]. Only tetramers bound with heme exhibit catalase activity. It has been shown that the N-terminus of catalase is essential for tetramer formation. In catalase of the yeast *Candida tropicalis*, deletion of the N-terminal four amino acids resulted in 80 % loss of catalase activity due to incomplete tetramer formation [Ueda et al., 2003]. In plants, catalase is the most important enzyme for scavenging H_2O_2 produced during photorespiration [Dat et al., 2000]. Furthermore, catalase is also im-

portant for maintaining the whole cell redox balance during oxidative stress and is indispensable for stress defence in C3 plants [Willekens et al., 1997]. Animals only possess one form of catalase, whereas plants have three different classes of catalases that differ in their expression patterns and physiological effects [Willekens et al., 1995]. Class I catalases show strong, light-dependent expression in leaves and are thought to be involved in scavenging H_2O_2 generated during photorespiration because they are very abundant in photosynthetically active cells. Class II catalases show strong, light-independent expression in vascular tissues and possibly play a role in lignification and stress responses [Dat et al., 2000, Orendi et al., 2001]. Class III catalases are mainly expressed in seeds and young seedlings and they break down H_2O_2 produced during fatty acid degradation [Dat et al., 2000, Willekens et al., 1995]. Catalases have been shown to be involved in the signal transduction pathways of various stress responses [Fukamatsu et al., 2003, Verslues et al., 2007].

In *Arabidopsis*, there are three catalase genes: class III catalase *CAT1*, class I catalase *CAT2* and class II catalase *CAT3* [Frugoli et al., 1996]. *CAT1* and *CAT3* are located consecutively on chromosome 1, and *CAT2* is located on chromosome 3. All three catalases consist of 492 amino acids and show high sequence similarity [Mhamdi et al., 2010]. *CAT2* and *CAT3* expression is regulated by circadian rhythm, with a morning-specific phase for *CAT2* and an evening-specific phase for *CAT3* [Zhong and McClung, 1996]. *CAT2* and *CAT3* expression is also dependant on the age of the plant. CAT2 activity decreases when plants start to bolt, whereas CAT 3 activity increases with age and senescence [Zimmermann et al., 2006, Zentgraf, 2009]. *CAT1* is expressed

in cotyledons of early seedlings and during later stages of senescence. *CAT2* is expressed predominantly in the mesophyll of green leaves, whereas *CAT3* is expressed in vascular tissue as well as in root tips and shoot meristem [Hu et al., 2010, Zimmermann et al., 2006]. *CAT2* and *CAT3* show high basal levels of expression, whereas basal expression of *CAT1* is weak but significantly increases under stress conditions [Du et al., 2008]. In *cat1*, *cat2*, *cat3*, and *cat2 cat3* T-DNA lines, total catalase activity has been reported to be decreased by 8 %, 76 %, 27 %, and 93 %, respectively [Hu et al., 2010]. In another study, where RNAi lines were used for *CAT1* and *CAT3* and a T-DNA line for *CAT2*, a decrease of 8 %, 83 %, and 55 % was reported for *cat1*, *cat2*, and *cat3*, respectively [Du et al., 2008]. Seven different isoforms of catalase tetramers have been identified in *Arabidopsis*, three consisting of only CAT1, CAT2 or CAT3, respectively, as well as three heterotetramers consisting of CAT1 and CAT2, and one heterotretamer consisting of CAT2 and CAT3 [Hu et al., 2010].

CAT2 plays a major role in scavenging H_2O_2 produced during photorespiration and *cat2* mutants are photorespiratory mutants when grown under standard irradiance, showing pale green colour, curled leaves, reduced size and plant fresh weight [Queval et al., 2007, Hu et al., 2010]. Under the same growth conditions, *cat1* and *cat3* exhibit wild type morphology, whereas the *cat2 cat3* double mutant shows a slightly more severe phenotype than *cat2* [Hu et al., 2010]. These symptoms only occur in the presence of photorespiration, *cat2* plants show wild type phenotype when grown under high CO_2 levels or when grown under irradiance that only allows slow rates of photorespira-

tion [Queval et al., 2007]. Increased H_2O_2 levels in unstressed *cat2* plants have been reported in some studies [Bueso et al., 2007, Hu et al., 2010] whereas in other cases increased H_2O_2 levels were only observed after infiltration with avirulent bacteria [Simon et al., 2010]. In *cat2* mutants H_2O_2 is processed through reductive H_2O_2 pathways, which require ascorbate and thiols, and results in a drastic increase of glutathione levels, but not generalised cellular oxidation [Queval et al., 2007]. Loss of CAT2 function seems to mimic processes that are triggered by pathogen infection, such as the hypersensitive response, and also has an impact on phytohormone signalling, such as ethylene and auxin signalling [Mhamdi et al., 2012]. It has been shown that the photorespiratory role of CAT2 is determined by its promoter activity and its 3'-untranslated region (UTR) [Hu et al., 2010]. The amino acid sequences of the three catalases are very similar, but their 3'-UTRs are highly divergent. Expression of *CAT2* driven by the *CAT2* promoter can restore wild type phenotype in the *cat2* mutant, whereas expression of *CAT2* driven by the *CAT1* or *CAT3* promoter does not. *CAT2* promoter driven expression of *CAT3* however, restores wild type phenotype, unlike expression of *CAT1* which also requires replacement of the *CAT1* 3'-UTR with that of *CAT2*. At bolting, *CAT2* is downregulated and APX1 activity increases, and a H_2O_2 peak can be observed [Zimmermann et al., 2006]. However, APX1 is not down-regulated on a transcriptional level. A feedback amplification loop has been proposed to explain the processes occuring during bolting. *CAT2* down-regulation is the initial step, which then leads to an increase in H_2O_2 levels and inactivation of APX1, which further increases H_2O_2 levels. These increased H_2O_2 levels lead to the induction of CAT3 activity, which then lowers H_2O_2 levels and restores

APX1 activity. CAT1 becomes detectable at later stages of plant development. The scenescence-associated transcription factor WRKY53 can be induced by H_2O_2 treatment, so the distinct H_2O_2 peak observed at bolting probably plays an important role in inducing a coordinated scenescence process.

CAT3 activity, but not the activity of other antioxidant enzymes, increases in response to oxidative stress [Orendi et al., 2001]. This response is suppressed once plants progress through a certain stage of senescence. Both *CAT2* and *CAT3* expression are increased, so there is regulation on a post-transcriptional level. *CAT3* expression also increases during sucrose starvation, possibly to compensate for additional H_2O_2 production caused by the use of alternative catabolic substrates by enzymes such as ACX4 [Contento and Bassham, 2010]. Salt overly sensitive 2 (SOS2) interacts with CAT2 and CAT3 in salt-stressed plants, but not in unstressed plants. SOS2 kinase activity is not required for the interaction. CAT3 shows a particularly strong interaction with SOS2, whereas no interaction between CAT1 and SOS2 was observed [Verslues et al., 2007]. Interestingly, SOS2 is located in the cytoplasm, so there is a possibility that CAT2 and CAT3 can also be found in the cytoplasm and not just in peroxisomes. All three catalases interact with Nucleotide diphosphate kinase 1 (NDK1), which is a cytosolic enzyme catalysing the transfer of a phospate group from nuceloside triphospate to nucleoside diphosphate [Fukamatsu et al., 2003]. NDK1 is very similar to NDK-P1, a 18 kDA protein in *Pisum sativum*, which is involved in photosignalling and shows increased phosphorylation after red light irradiation. Calmodulin, a calcium-binding protein, interacts with CAT3 *in vitro* in a Ca^{2+}-dependent

manner [Yang and Poovaiah, 2002], and CAT3 was also shown to be activated by Ca^{2+} *in vivo* [Costa et al., 2010, Costa et al., 2013].

6.2 Other ROS-associated enzymes and antioxidants

The phytoalexin-deficient mutant *pad2* shows increased susceptibility to a wide range of pathogens and herbivorous insects [Dubreuil-Maurizi et al., 2011]. The *pad2* mutation is located in γ-glutamylcysteine synthetase, which is involved in the first step of glutathione (GSH) biosynthesis [Parisy et al., 2007]. As a result, *pad2* mutants only contain about 20 % of wild type glutathione levels, and the glutathione redox potential is less reducing [Dubreuil-Maurizi et al., 2011]. *pad2* plants show very low *PR1* expression and SA levels in response to pathogen challenge, the latter being caused by a lack of pathogen-triggered *ICS1* expression. Furthermore, *pad2* plants are also impaired in H_2O_2 and NO production during pathogen infection, exhibit reduced HR, and have decreased levels of camalexin and indolate glucosinolates.

Thioredoxins (Trx) break down GSNO and can also directly denitrosylate SNO proteins [Benhar et al., 2009]. There are 42 *Trx* genes in *Arabidopsis* [Meyer et al., 2005] and Trx are present in a variety of different plant tissues [Vieira Dos Santos and Rey, 2006]. Cytosolic Trx-h proteins are thought to be involved in redox regulation in vascular tissues [Reichheld et al., 2002]. There are eight *Arabidopsis Trx-h* genes, with *Trx-h3* showing by far the highest expression levels. *Trx-h5* expression is strongly induced by pathogen infection,

whereas *Trx-h3* expression does not change after pathogen challenge.

Seven ascorbate-deficient *vtc* mutants have been identified [Colville and Smirnoff, 2008], with the seven mutations being located in four different genes. Out of these four loci, *vtc1* and *vtc2* result in the most drastic decrease in ascorbate levels, with plants containing only 25 to 30 % of wild type ascorbate levels. All mutants show wild type growth, little change in other parts of the antioxidant system and no severe oxidative stress. However, transcript levels of *PR* genes, cell wall peroxidase activity, and camalexin accumulation are increased [Colville and Smirnoff, 2008], resulting in increased resistance to infection by virulent pathogens and higher SA levels in *vtc1* and *vtc2* plants [Barth et al., 2004, Pavet et al., 2005]. All four mutants are more salt-sensitive than wild type plants [Huang et al., 2005], and *vtc1* and *vtc2* are extremely sensitive to ozone, while *vtc3* and *vtc4* are only slightly more sensitive than wild type [Conklin et al., 2000]. *vtc1* and *vtc2* also exhibit reduced thermotolerance [Larkindale et al., 2005], and *vtc2* shows reduced acclimation to high light, showing signs of oxidative stress such as lipid peroxidation and bleaching and 30% higher GSH levels than wild type under high light [Mueller-Moule et al., 2003].

6.3 Loss of various ROS-related genes does not suppress the *atgsnor1-3* phenotype

To confirm that the phenotype of the suppressor mutants was indeed caused by a loss of CAT3 activity, *atgsnor1-3 cat3* double mutants were produced.
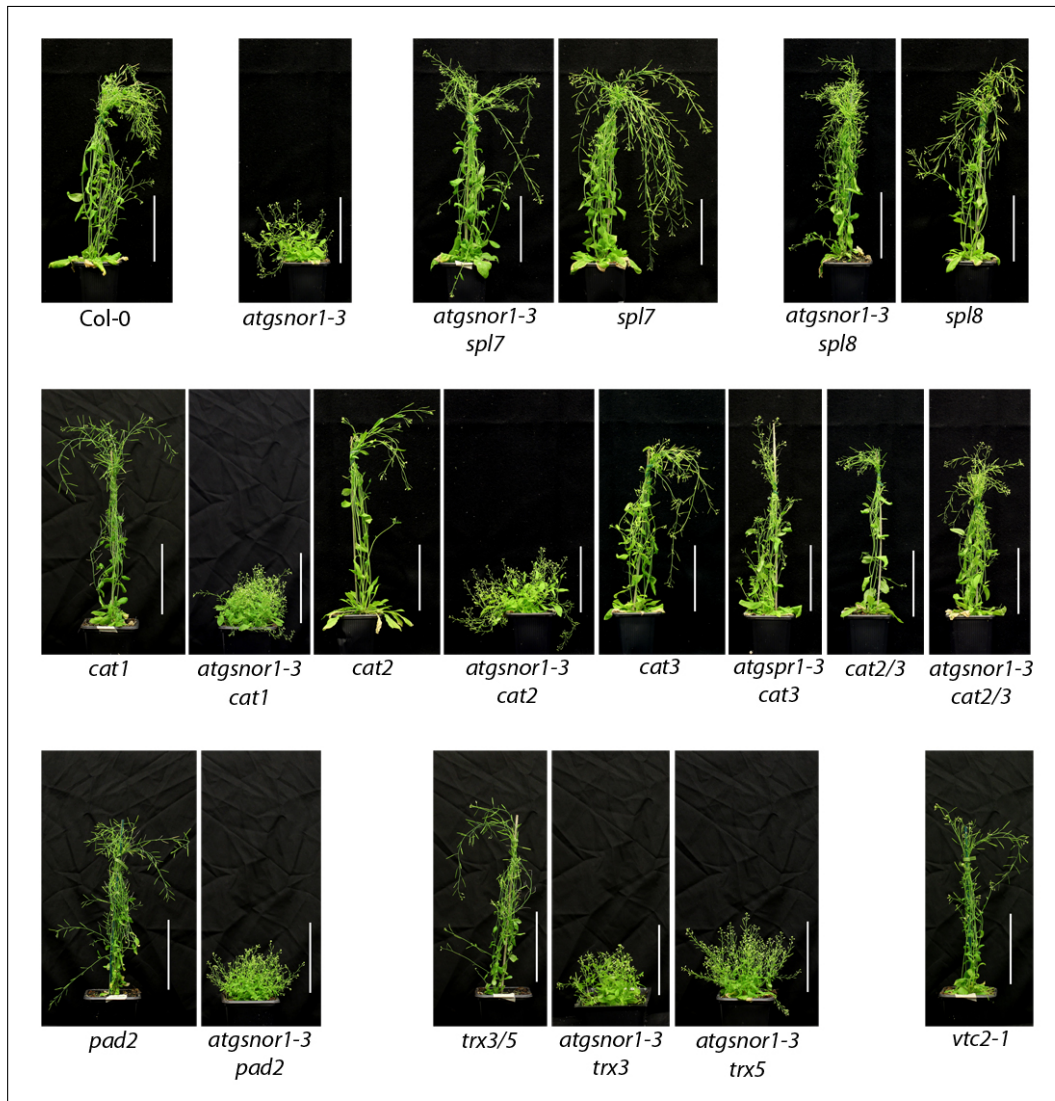
Figure 6.1: 9 week old *Arabidopsis* plants. The effects of loss-of-function of various ROS-related genes in wild type (Col-0) and *atgsnor1-3* background. Col-0, *atgsnor1-3*, *atgsnor1-3 spl7*, *spl7*, *atgsnor1-3 spl8*, and *spl8* are included for comparison. Scale bars = 10cm.

atgsnor1-3 cat1, *atgsnor1-3 cat2*, and *atgsnor1-3 cat2 cat3* mutants were also obtained, to investigate whether loss of other catalases also suppresses the *atgsnor1-3* phenotype. It is not possible to produce a *cat1 cat2 cat3* triple mutant using T-DNA lines because *CAT1* and *CAT3* are located consecutively on chromosome 1. Loss of CAT3 activity presumably disturbs the intracellular redox status; so it was tested whether disturbing the redox status through loss-of-function of other redox-related genes, namely *pad2*, *vtc2-1*, *trx3*, and *trx5*, would also have an effect on growth morphology and possibly restore apical dominance in *atgsnor1-3*. *atgsnor1-3 pad2*, *atgsnor1-3 trx3*, and *atgsnor1-3 trx5* double mutants were produced. No *atgsnor1-3 trx3 trx5* triple mutants or *atgsnor1-3 vtc2-1* double mutants have been identified so far.

atgsnor1-3 cat3 and *atgsnor1-3 cat2 cat3* plants showed wild type shoot morphology, while *atgsnor1-3 cat1* and *atgsnor1-3 cat2* had not recovered apical dominance and showed bushy stunted growth [Fig. 6.1]. *cat1*, *cat2*, *cat3*, and *cat2 cat3* showed wild type shoot morphology when grown under moderate lighting conditions. *pad2*, *trx3 trx5* and *vtc2-1* also exhibited wild type growth, while *atgsnor1-3 pad2*, *atgsnor1-3 trx3*, and *atgsnor1-3 trx5* showed *atgsnor1-3* phenotype [Fig. 6.1].

6.4 Loss of CAT3, but not CAT2, restores wild type HR in *atgsnor1-3*

atgsnor1-3 cat2, *atgsnor1-3 cat3*, and *atgsnor1-3 cat2 cat3* plants were inoculated with *Pst* DC3000 *avrB* and leaves were stained with Trypan Blue 24

hours post inoculation (hpi) to visualise cell death. Col-0 and *atgsnor1-3* were included as controls. Just like the suppressor mutants, *atgsnor1-3 cat3* showed wild type HR [Fig. 6.2]. *atgsnor1-3 cat2* and *atgsnor1-3 cat2 cat3* showed increased HR, and were not significantly different from *atgsnor1-3* [Fig. 6.2].
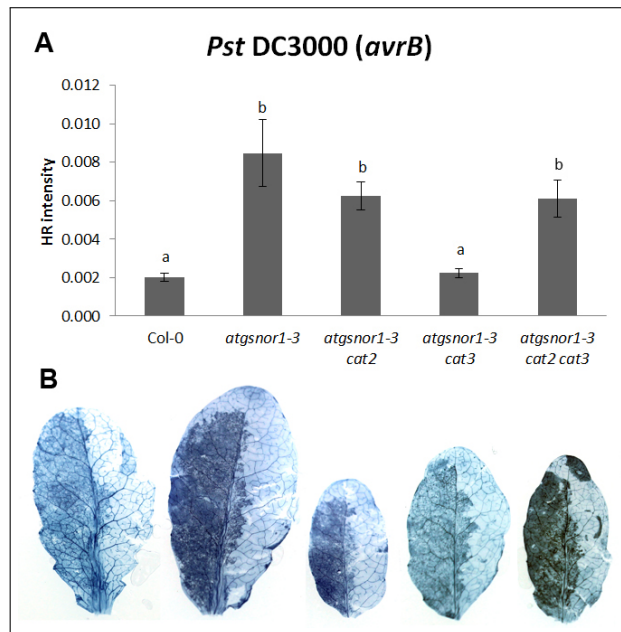
Figure 6.2: (A) HR intensity 24 hours post inoculation (hpi) with 10^7 CFU ml^{-1} *Pst* DC3000 (*avrB*). Error bars show SE (n=4). Values with different letters are significantly different (P<0.01, Duncan's MRT). (B) Trypan Blue stained *Arabidopsis* leaves 24 hours after inoculation with 10^7 CFU ml^{-1} *Pst* DC3000 (*avrB*). The left half of each leaf was inoculated, the right half was left untreated. From left to right: Col-0, *atgsnor1-3*, *atgsnor1-3 cat2*, *atgsnor1-3 cat3*, *atgsnor1-3 cat2 cat3*.

6.5 Discussion

atgsnor1-3 cat3 double mutants showed wild type growth morphology. This was to be expected, considering that the suppressor mutants have a mutation in

CAT3 and show a loss of CAT3 activity [Fig. 4.15]. Interestingly, neither loss of *CAT1* nor *CAT2* suppressed the *atgsnor1-3* phenotype, and no difference was observed between growth morphology of *atgsnor1-3 cat3* and *atgsnor1-3 cat2 cat3* plants [Fig. 6.1]. It is intriguing that the suppression of *atgsnor1-3* phenotype appears to be specific to loss of CAT3 activity, and that CAT1 and CAT2 do not seem to have an effect.

Loss of CAT3 activity recovers apical dominance in the *atgsnor1-3* background. H_2O_2 has been shown to play a role in regulating shoot branching [Tognetti et al., 2010]. H_2O_2 levels in *atgsnor1-3* plants are decreased
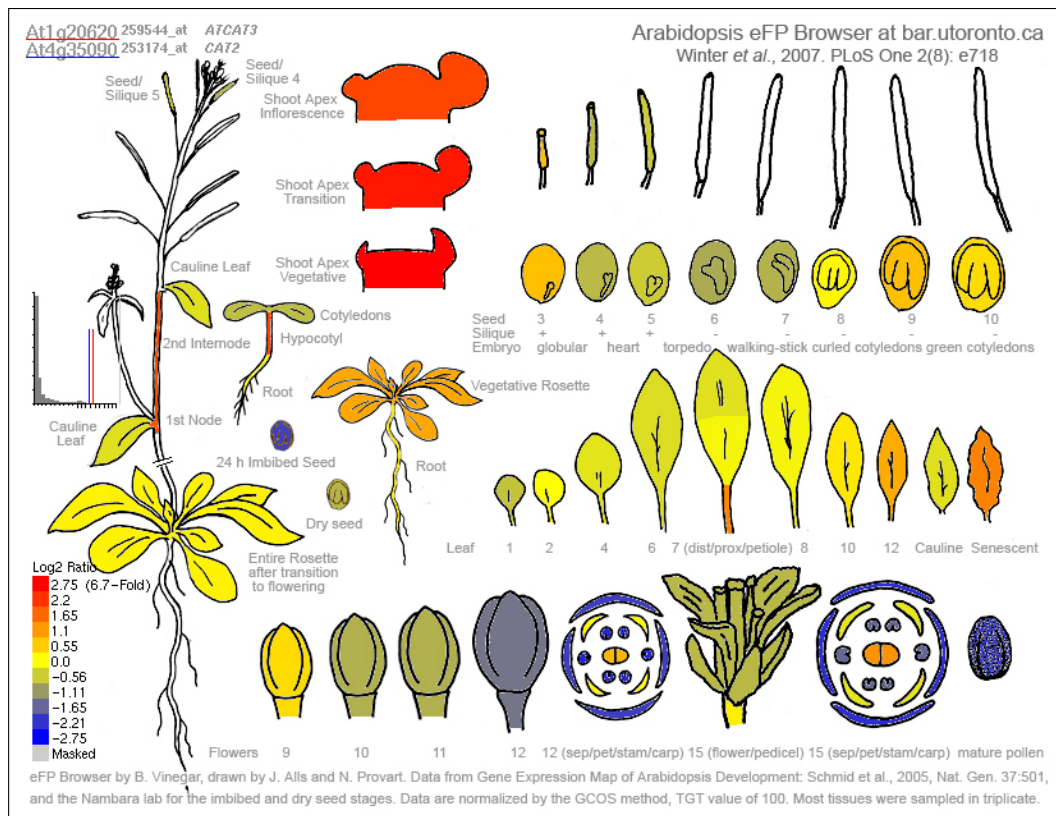
Figure 6.3: *CAT3* expression compared to *CAT2* expression. *Arabidopsis* eFP Browser, showing the tissues in which *CAT3* (red) or *CAT2* (blue) is predominantly expressed. [Winter et al., 2007]

due to loss of RbohD activity [Yun et al., 2011], but loss of CAT3 activity could possibly counteract this and increase H_2O_2 content to a level sufficient for restoration of apical dominance. Intriguingly, loss of CAT1 or CAT2 did not result in wild type shoot morphology in an *atgsnor1-3* background. CAT1 only accounts for less than 10% of total catalase activity [Hu et al., 2010, Du et al., 2008], so it is quite possible that loss of CAT1 activity simply does not have any noticeable effect on H_2O_2 levels. CAT2, on the other hand, is the major catalase in *Arabidopsis* [Hu et al., 2010, Du et al., 2008], so it might seem surprising that *atgsnor1-3 cat2* plants have not recovered apical dominance. However, while the three catalase are structurally very similar [Mhamdi et al., 2010], they differ in their temporal and spatial expression patterns [Hu et al., 2010, Zentgraf, 2009, Zimmermann et al., 2006]. *CAT3* is predominantly expressed in shoots, at a higher level than *CAT2* [Fig. 6.3]. Furthermore, *CAT3* is upregulated during bolting, while *CAT2* expression decreases [Zimmermann et al., 2006, Zentgraf, 2009]. Considering the different expression patterns of *CAT2* and *CAT3*, it could be that loss of CAT2 activity does not restore apical dominance simply because *CAT2* is not expressed at a high enough level in the right tissues and/or at the right time during development. It could also be speculated that loss of CAT2 activity would increase H_2O_2 levels too much, and that only loss of CAT3 activity results in H_2O_2 levels that are sufficiently increased to restore apical dominance, but not so much as to have a negative effect on development. However, this scenario is very unlikely because the *atgsnor1-3 cat2 cat3* triple mutant, which presumably has even higher H_2O_2 levels than *atgsnor1-3 cat2*, has recovered apical dominance. *atgsnor1-3 trx3*, *atgsnor1-3 trx5*, and *atgsnor1-3 pad2* plants did not recover apical dominance.

trx3 trx5 and *pad2* plants show wild type growth, but it is worth noting that a triple mutant (*ntra ntrb cad2*) with reduced levels of thioredoxin and glutathione showed loss of apical dominance, vascular defects, and reduced lateral roots [Bashandy et al., 2010, Bashandy et al., 2011]. Based on this, it can be speculated that in the *atgsnor1-3* background, *trx3*, *trx5*, or *pad2* would have a negative rather than a positive effect on plant development.

Only *atgsnor1-3 cat3* plants showed wild type HR, while *atgsnor1-3 cat2* and *atgsnor1-3 cat2 cat3* showed increased HR just like *atgsnor1-3*. The balance between H_2O_2 and NO is important for regulating HR [Delledonne et al., 2001]. It is possible that H_2O_2 levels in *atgsnor1-3 cat3* are increased just enough to result in the correct ratio of H_2O_2 and NO, while in *atgsnor1-3 cat2* and *atgsnor1-3 cat2 cat3* the H_2O_2 levels are too high. It would be useful to repeat this experiment with *atgsnor1-3 cat1* plants and also include *cat1*, *cat2*, *cat3*, and *cat2 cat3* plants for comparison.

Loss of other redox-related genes, namely *pad2*, *trx3*, and *trx5*, did not restore wild type shoot morphology in the *atgsnor1-3* background, which further suggests that suppression of the *atgsnor1-3* phenotype is quite possibly specific to *cat3* only. Several hundred F_2 plants were genotyped in an attempt to obtain *atgsnor1-3 trx3 trx5* triple mutants, but without success. Considering the number of plants used it would have been expected to identify some triple mutants, so it is well possible that *atgsnor1-3 trx3 trx5* plants are not viable. While loss of several redox-related genes did not restore wild type shoot morphology in *atgsnor1-3* plants, it remains to be investigated whether they suppress other aspects of the *atgsnor1-3* phenotype, such as increased

HR or pathogen susceptibility. *pad2* plants only have about 20% of wild type glutathione levels, and thioredoxins break down GSNO and denitrosylate proteins, which could lead to decreased SNO levels in *atsgnor1-3 pad2*, *atgsnor1-3 trx3* and *atgsnor1-3 trx5* plants compared to *atgsnor1-3* plants.

Chapter 7

General discussion and future work

7.1 Loss of CAT3 activity restores apical dominance in *atgsnor1-3*

atgsnor1-3 plants have lost apical dominance, develop a high number of lateral shoots and show stunted growth [Feechan et al., 2005]. Auxin regulates shoot branching and a disruption of polar auxin transport leads to increased branching and a loss of apical dominance [Leyser, 2003]. In addition to regulating shoot branching, auxin also plays an important role in various other stages of plant development, such as lateral root formation or vascular development [Woodward and Bartel, 2005]. The *atgsnor1-3* phenotype is reminiscent of the phenotype of mutants impaired in polar auxin transport, such as *bushy and dwarf 1* (*bud1*), *pin-formed 1* (*pin1*), and *transport inhibitor response 3*

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(*tir3*). The *bud1* mutant has lost apical dominance, and shows highly branched and dwarfed growth [Dai et al., 2006]. This phenotype is caused by overexpression of *MKK7*, which is a negative regulator of polar auxin transport. The *pin1* mutant develops pin-like structures instead of flower buds and has defects in vascular development [Gälweiler et al., 1998, Okada et al., 1991]. The *tir3* mutant shows reduced apical dominance and decreased height, as well as decreased petiole and root length, and also fewer and shorter siliques [Gil et al., 2001, Ruegger et al., 1997]. It can be speculated that the developmental phenotype of *atgsnor1-3* is caused by disruptions in polar auxin transport and/or auxin signalling, possibly through S-nitrosylation of auxin-related proteins [Fig. 7.1]. High levels of NO inhibit auxin transport in primary roots, which leads to reduced primary root growth [Fernández-Marcos et al., 2011]; and it has been shown that the *Arabidopsis* TIR1 auxin receptor is a target for S-nitrosylaton [Terrile et al., 2012].

There is also crosstalk between auxins and ROS; auxins can regulate ROS homeostasis and vice versa [Tognetti et al., 2012]. Exogenous auxin treatment of *Arabidopsis* seedlings induces ROS production [Peer and Murphy, 2006], and ROS have also been shown to regulate auxin homeostasis [Tognetti et al., 2010]. Among the ROS, H_2O_2 plays a particularly important role and has been implicated in a variety of physiological processes, including plant growth and development [Foreman et al., 2003]. The TIR1/AFB auxin receptors regulate H_2O_2 levels and antioxidant enzymes [Iglesias et al., 2010]. NADPH oxidases also seem to be crucial for auxin signalling. The NADPH oxidase RbohD is induced by auxin treatment and contributes to auxin-induced ROS produc-

tion [Peer et al., 2013], and loss of RbohC results in defects in root growth, namely short root hairs and stunted roots [Foreman et al., 2003]. In maize, auxin-induced growth can be inhibited by ROS scavengers or by NADPH oxidase inhibitors [Schopfer et al., 2002]. In *atgsnor1-3* plants, RbohD is S-nitrosylated, rendering it inactive and thereby decreasing ROS levels [Yun et al., 2011]. In addition to possibly perturbed auxin signalling and/or transport, reduced ROS levels could also contribute to the growth defects observed in *atgsnor1-3* plants [Fig. 7.1].
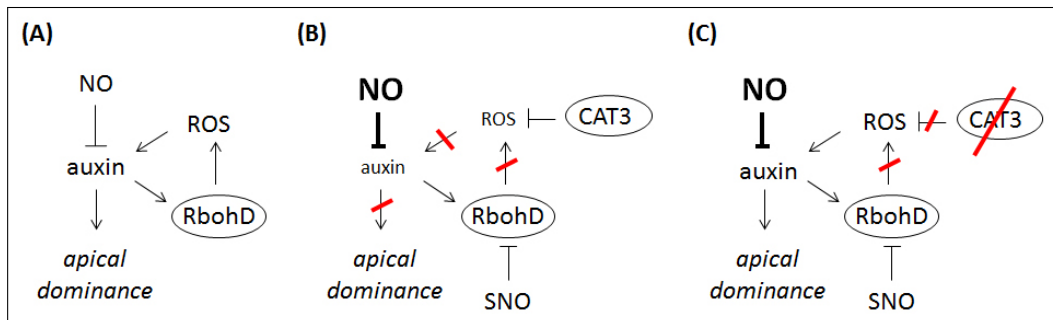
Figure 7.1: Loss of CAT3 activity restores apical dominance in *atgsnor1-3*. (A) Wild type *Arabidopsis*. Auxin is involved in regulating shoot branching; NO negatively regulates auxin levels, while ROS positively regulate auxin levels; auxin positively influences ROS levels through its induction of RbohD. (B) *atgsnor1-3* plants. Increased NO levels negatively impact auxin levels; RbohD activity is blunted by S-nitrosylation, leading to a decrease in ROS levels and further decrease of auxin levels; apical dominance is lost due to decreased auxin levels. (C) Suppressor mutants. Loss of CAT3 activity leads to increased ROS levels and subsequent increase in auxin levels; apical dominance is restored due to normalised auxin levels.

7.2 Loss of CAT3 activity restores wild type HR in *atgsnor1-3*

atgsnor1-3 plants show increased HR in response to inoculation with avirulent bacteria [Yun et al., 2011]. The balance between NO and H_2O_2 is important for regulating cell death [Delledonne et al., 2001]. During HR, $O_2^{\bullet-}$ is produced by NADPH oxidase, which is then dismutated to H_2O_2 by SOD [Delledonne et al., 2001, Overmyer et al., 2003]. In soybean cell cultures both NO and H_2O_2 are necessary for inducing cell death, and an increase of either NO or H_2O_2 is not sufficient [Delledonne et al., 2001]. In *Arabidopsis* this seems to be slightly different. High SNO concentrations, as observed in *atgsnor1-3* or *nox1*, positively regulate HR, even when ROS levels are reduced [Yun et al., 2011]. Based on these results it can be speculated that in *Arabidopsis* the ratio between NO and H_2O_2 is important for inducing HR cell death of the appropriate magnitude, but an increase in NO levels leads to increased cell death rather than abolishising it [Fig. 7.2]. It could be that there are differences in HR regulation between *Arabidopsis* and soybean, or perhaps more likely, that the use of cell cultures is not a suitable method for studying cell death. In addition to increased SNO levels, the NADPH oxidase RbohD is S-nitrosylated in *atgsnor1-3*, which abolishes its ability to synthesis $O_2^{\bullet-}$ [Yun et al., 2011]. This leads to decreased ROS levels in *atgsnor1-3*, which further disturbs the NO to H_2O_2 balance.

atgsnor1-3 spl7 and *atgsnor1-3 spl8* have recovered wild type HR. Loss of CAT3 activity due to the *spl7* and *spl8* mutations presumably leads to an in-

crease in H_2O_2 levels, which leads to a more balanced NO to H_2O_2 ratio and as a result, wild type HR [Fig. 7.2]. Not surprisingly, *atgsnor1-3 cat3* also showed wild type HR, but *atgsnor1-3 cat2* and *atgsnor1-3 cat2 cat3* showed increased HR even though they presumably have higher H_2O_2 levels than *atgsnor1-3 cat3*. It could be that loss of CAT3 activity leads to just enough H_2O_2 accumulation to restore a wild type NO to H_2O_2 balance, wheras loss of CAT2 or loss of CAT2 and CAT3 results in too much H_2O_2 accumulation, thereby shifting the NO to H_2O_2 ratio too far towards H_2O_2. It has been reported that *cat2* plants develop lesions when grown under high light conditions [Queval et al., 2007, Hu et al., 2010], so it is possible that not only elevated NO levels
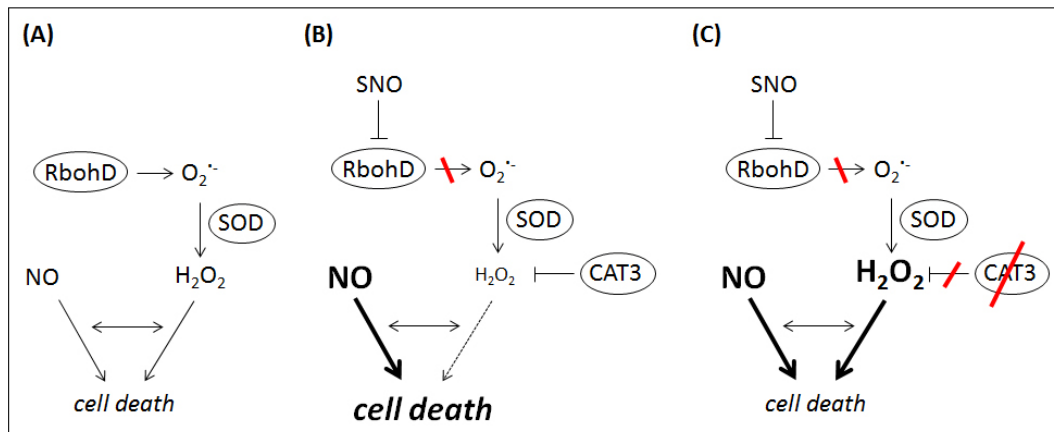
Figure 7.2: Loss of CAT3 activity restores wild type HR in *atgsnor1-3*. (A) Wild type *Arabidopsis*. RbohD produces $O_2^{\bullet-}$, which is then metabolised to H_2O_2 by SOD; NO and H_2O_2 together regulate HR, a balanced production of both is necessary (indicated by double sided arrow) (B) *atgsnor1-3* plants. NO levels are increased; RbohD activity is blocked by S-nitrosylation, leading to a decrease in $O_2^{\bullet-}$ production and subsequent decrease of H_2O_2 levels; increased NO levels together with decreased H_2O_2 levels cause increased cell death. (C) Suppressor mutants. NO levels are still increased; H_2O_2 are also increased due to a loss of CAT3 activity, this leads to a more balanced ratio of NO and H_2O_2, and wild type cell death.

but also elevated ROS levels lead to increased cell death, which could explain
the enhanced HR observed in *atgsnor1-3 cat2* and *atgsnor1-3 cat2 cat3*.

7.3 Loss of CAT3 activity partially restores disease resistance in *atgsnor1-3*

atgsnor1-3 spl7 and *atgsnor1-3 spl8* are still susceptible to virulent pathogens.
SA plays an important role in disease signalling after infection with *Pst* DC3000
and plants that are deficient in SA accumulation are more susceptible to viru-
lent and avirulent pathogens [Durner et al., 1997, Nawrath and Metraux, 1999].
Basal SA levels in the suppressor mutants as well as SA levels after inoculation
with *Pst* DC3000 are very low, just as in *atgsnor1-3* [Sorhagen, 2010], so it
is possible that the suppressor mutants' susceptibility to virulent pathogens is
caused by the reduced SA levels.

The suppressor mutants are more resistant to *Pst* DC3000 (*avrB*), *Pst*
DC3000 (*avrRpm1*), and *Pst* DC3000 (*avrRps4*) than *atgsnor1-3*, but not as
resistant as wild type plants. The effectors avrB and avrRpm1 are recognised
by the CC-NB-LRR R protein RPM1, while avrRps4 is recognised by the TIR-
NB-LRR R protein RPS4 [Bisgrove et al., 1994, Hinsch and Staskawicz, 1996].
Signalling downstream of CC-NB-LRR R proteins requires NDR1, while sig-
nalling downstream of TIR-NB-LRR R proteins is dependent on EDS1 [Aarts
et al., 1998]. After inoculation with *Pst* DC3000 (*avrB*), SA levels in the
suppressor mutants remain low [Sorhagen, 2010], so it can be ruled out that
SA-dependent disease signalling is responsible for the partial resistance of the

suppressor mutants. However, SA-independent signalling in response to avirulent pathogens has been reported, and this could be a possible explanation for partial recovery of disease resistance to avirulent *Pst* DC3000 in the suppressor mutants.

Two different pathways are involved additively in signalling downstream of RPM1; one pathway involves HR-associated signals and is NPR1-independent, while the other one is SA-dependent and requires NPR1 [Zhang and Shapiro, 2002]. *ndr1* plants are impaired in SA accumulation but still show *PR1* expression and SAR in response to *Pst* DC3000 (*avrB*), but to a lesser extent than wild type plants [Shapiro and Zhang, 2001]. Furthermore, *nahG* plants, which cannot accumulate SA, are still able to mount RPM1-mediated defence responses, but are impaired in RPS2-mediated responses [Tao et al., 2003]. *sid* mutants do not accumulate SA after pathogen infection and show reduced *PR1* expression, but *PR2* and *PR5* are expressed normally [Nawrath and Metraux, 1999]. These results show that, while SA is needed for full induction of the defence response, SA-independent pathways also contribute to plant disease resistance. The SA-independent pathway downstream of RPM1 is dependent on HR-associated signals, so it is quite likely that NO, ROS or both are involved. Given the extreme disease susceptibilty of *atgsnor1-3* plants, it seems likely that both the SA-dependent pathway as well as the SA-independent pathway are impaired, the latter possibly due to perturbed NO and/or ROS levels. In the suppressor mutants, loss of CAT3 function presumably leads to an increase in ROS levels, thereby at least partially restoring the redox balance, which makes it possible for the SA-independent signalling pathway
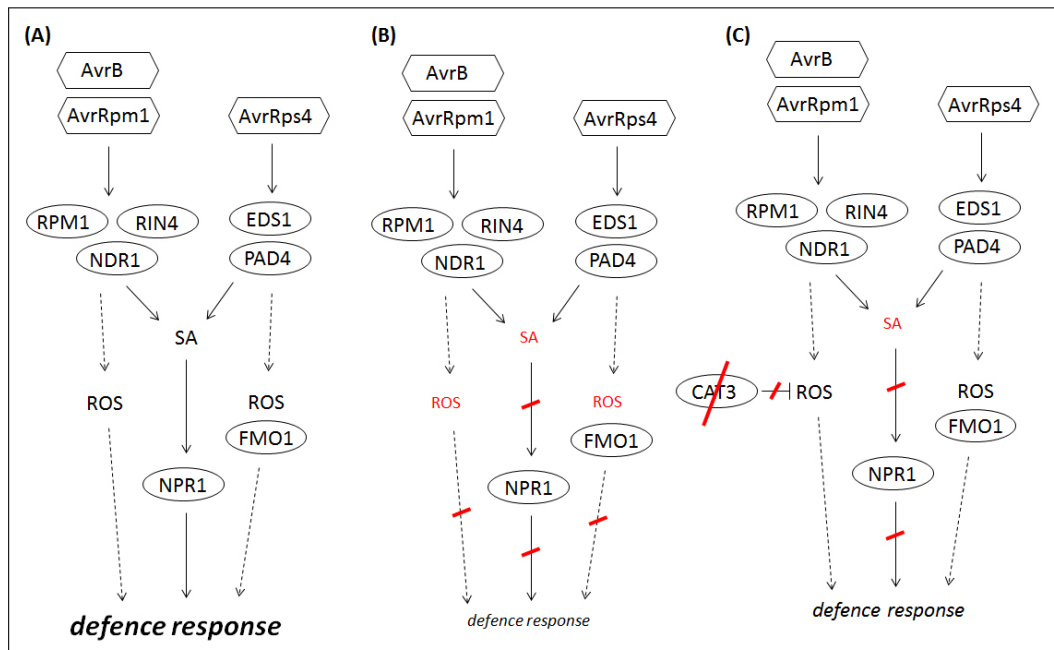
Figure 7.3: Loss of CAT3 activity partially restores disease resistance in *atgsnor1-3*. (A) Wild type *Arabidopsis.* Recognition of the TTEs AvrB and AvrRpm1 involves RPM1, RIN4, and NDR1; signalling downstream of the TTE AvrRps4 is regulated by EDS1 and PAD4; after pathogen recognition SA levels increase and NPR1 translocates to the nucleus where it induces defence gene expression; ROS-dependent but SA-independent pathways (indicated by dashed lines) are possibly also involved in disease resistance; SA-dependent and ROS-dependent pathways might additively. (B) *atgnor1-3* plants show decreased SA and ROS levels, and are impaired in both SA-dependent and ROS-dependent disease signalling. (C) The suppressor mutants are still impaired in SA-dependent signalling but have recovered ROS-dependent signalling.

to be activated. However, since the suppressor mutants are still impaired in SA signalling and accumulation, the SA-dependent pathway remains inactive, which explains why the suppressor mutants have only partially restored disease resistance [Fig. 7.3].

EDS1 has been shown to be involved in SA-independent signalling [Bartsch

et al., 2006]. Flavin-dependent monooxygenase (FMO1) positively regulates the SA-independent branch of EDS1 signalling, while the cytosolic Nudix hydrolase NUDT7 acts as a negative regulator [Bartsch et al., 2006]. FMO1 is also required for full TIR-NB-LRR dependent resistance to avirulent pathogens [Bartsch et al., 2006], perhaps indicating that the SA-dependent and SA-independent pathways act additively. Both EDS1 as well as FMO1 and NUDT7 have been shown to be connected to ROS signalling, indicating that the SA-independent branch of the EDS1 signalling pathway could be ROS-dependent. FMOI transcription was shown to be upregulated in response to superoxide but not by hydrogen peroxide or ozone [Olszak et al., 2006]. NUDT7 hydrolyses ADP-ribose and NADH *in vitro* [Ogawa et al., 2005]. In mammalian cells, ADP-ribose acts as a second messenger in oxidative stress-induced ion channel activation and apoptosis [Perraud et al., 2001, Perraud et al., 2005, Kolisek et al., 2005], and it is possible that it could fulfill a similar function in plant cells. The potential role for NUDT7 could be to regulate ADP-ribose levels as well as the NADH/NAD$^+$ ratio [Ogawa et al., 2005]. NUDT7 was among the upregulated genes in *Arabidopsis* mutants lacking cytosolic APX1, which turns over H$_2$O$_2$ under light stress [Davletova et al., 2005], further linking NUDT7 with ROS signalling. EDS1 has been shown to transduce ROS-derived signals in biotic and abiotic stress signalling [Rustérucci et al., 2001, Mateo et al., 2004, Mühlenbock et al., 2008, Straus et al., 2010]. EDS1 regulates signalling in response to chloroplast-derived O$_2$$^{\bullet-}$, and facilitates SA-assisted H$_2$O$_2$ accumulation to limit cell death [Straus et al., 2010]. It could be that both SA-dependent and SA-indepedent EDS1 signalling pathways are impaired in *atgsnor1-3* due to decreased SA levels and perturbed redox balance, but that

loss of CAT3 activity in the suppressor mutants leads to an increase in ROS levels which then restores the SA-independent EDS1 signalling pathway [Fig. 7.3]. However, since both pathways might act additively, activation of the SA-independent pathway is only enough to partially restore disease resistance. The suppressor mutants appeared to show higher *PR1* expression than *atgsnor1-3* but less than wild type plants, which could be due to activation of the SA-independent EDS1 pathway.

In addition to SA and ROS, auxin might also play a role in disease resistance. Auxin has been shown to promote susceptibility to biotrophic pathogens [Robert-Seilaniantz et al., 2007], and repression of auxin signalling restricts *Pst* DC3000 growth [Navarro et al., 2006]. Infection with virulent pathogens such as *Pst* DC3000 leads to increased auxin levels [O'Donnell et al., 2003b], and it has been shown that *Pst* DC3000 induces genes involved in auxin biosynthesis [Schmelz et al., 2003]. The type III effector AvrRpt2 has been show to alter auxin physiology [Chen et al., 2007], and the type III effector AvrBs3 induces auxin-responsive genes [Marois et al., 2002]. Furthermore, auxin biosynthesis and transport mutants are impaired in SAR. They show reduced levels of free and conjugated SA, and increased levels of JA and ABA in response to infection with *Pst* DC3000 (*avrRpm1*) [Truman et al., 2010]. SA inhibits auxin signalling by repressing auxin-related genes, including TIR1 [Wang et al., 2007]. The *atgsnor1-3* mutant is impaired in SA biosynthesis and signalling [Feechan et al., 2005]. It could be speculated that because of its low SA levels the *atgsnor1-3* plants might have increased auxin levels, which would negatively impact disease resistance. However, as previ-

ously discussed, it is likely that the *atgsnor1-3* mutant is impaired in polar auxin transport and/or auxin signalling, so increased auxin levels might not necessarily play a role in the mutant's disease susceptibility.

7.4 Future work

A suppressor screen uncovered two suppressor mutations of *atgsnor1-3*, which were both identified as mutations in CAT3. Intriguingly, even though the suppressor mutants still show reduced SA levels, and also appear to still have high SNO levels, they have recovered apical dominance and show wild type HR, and have partially recovered disease resistance to avirulent pathogens. This points towards the involvement of SA-independent and ROS-dependent signalling pathways, but the exact mechanisms remain yet to be uncovered and many open questions remain.

The suppressor mutants are still susceptible to GNSO and H_2O_2, and are resistant to the superoxide donor MV, just like *atgsnor1-3*. Based on these results it is very likely that SNO levels in the suppressor mutants are still high. However, this only relates to basal SNO levels, and it could be that SNO levels after pathogen infection are slightly lower in the suppressor mutants than in *atgsnor1-3*. This scenario might be unlikely because SA levels in the suppressor mutants after infection with virulent and avirulent bacteria remain low; but nevertheless it would be necessary to measure SNO levels in the suppressor mutants both when unchallenged and after infection with pathogens.

Based on the total catalase levels of the suppressor mutants compared

to *cat3* plants, it was speculated that the *spl7* and *spl8* mutations lead to a complete loss of CAT3 activity. To test whether this is really the case, the mutant CAT3 proteins could be expressed recombinantly and tested for catalase activity. However, expression of bioactive plant catalase is complicated [Ray et al., 2012], so this might not be a feasible approach. Furthermore, CAT3 has been shown to interact with NDK1 and SOS2 [Verslues et al., 2007], and it could be tested if this interaction still takes place with the mutant CAT3 proteins. It is possible that the *spl7* and *spl8* mutations might impair correct protein folding of CAT3, and Western blots could be used to test whether this is the case.

It was presumed that only loss of CAT3, and not CAT1 or CAT2, can restore apical dominance in *atgsnor1-3* because of the temporal and spatial expression pattern of CAT3. However, while the suppressor mutants have recovered apical dominance, they still have very short roots and small siliques. It would be interesting to test whether the *atgsnor1-3 cat2 cat3* mutant shows a different growth phenotype and has perhaps longer roots or bigger siliques than the suppressor mutants.

atgsnor1-3 2x35S::CAT3 plants have lost apical dominance, but do not show stunted growth. The *atgsnor1-3 2x35S::CAT3* plants uncouple loss of apical dominance from stunted growth, and could therefore be quite useful as a new tool to study the effects of *atgsnor1-3* in different phenotypes. Further characterising the *atgsnor1-3 2x35S::CAT3* plants, especially looking at HR and disease resistance, could possibly give more insight into the interaction between *atgsnor1-3* and different levels of *CAT3* expression.

The suppressor mutants and *atgsnor1-3 cat3* have restored wild type HR, while *atgsnor1-3*, *atgsnor1-3 cat2*, and *atgsnor1-3 cat2 cat3* show increased HR. It would be useful to also test HR of *cat1*, *cat2*, *cat3*, *cat2 cat3*, and *atgsnor1-3 cat1*, to disentangle the contributions of NO and H_2O_2 to HR intensity. *cat2* and *cat2 cat3* show lesion development when grown under high light conditions [Queval et al., 2007, Hu et al., 2010], so it can be speculated that they might also show increased HR after pathogen infection. HR in *cat2* and *cat2 cat3* might even be higher than in *atgsnor1-3 cat2* and *atgsnor1-3 cat2 cat3*, because of a more balanced NO to H_2O_2 ratio in the latter mutants. *atgsnor1-3* plants have been shown to be as resistant as wild type plants to the avirulent oomycete *Hyaloperonospora arabidopsidis* isolate Emwa1, due to increased cell death [Yun et al., 2011]. It would be interesting to test if the suppressor mutants are still resistant to *H. arabidopsidis*, even though they show wild type HR.

It was speculated that loss of CAT3 activity leads to increased H_2O_2 levels in the suppressor mutants. However, to confirm this it would necessary to quantify H_2O_2 levels. This could be done by staining with 3,3-diaminobenzidine (DAB), which forms precipitates when exposed to H_2O_2 [Thordal-Christensen et al., 1997]. However, DAB staining was tested repeatedly without satisfying results, so an alternative method might be advisable. Another option would be to infiltrate leaves with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), and then expose to 365 nm of UV light [Wolfe et al., 2000]. Quantifying $O_2^{\bullet-}$ levels could also be useful, this can be done by staining with nitroblue tetrazolium (NBT) [Jabs et al., 1996].

Even though SA levels in the suppressor mutants remain low, they have partially recovered disease resistance to avirulent bacteria, which points towards the involvement of SA-independent disease signalling pathways. As mentioned previously, signalling downstream of both CC-NB-LRR and TIR-NB-LRR proteins has been shown to be regulated through both SA-independent and SA-dependent pathways. To further test if SA-independent signalling is responsible for partial recovery of disease resistance, it could be checked whether the suppressor mutants are susceptible to *Pst* DC3000 *avrRpt2*. The disease response against *Pst* DC3000 *avrRpt2* seems to be much more dependent on SA signalling than the response to *Pst* DC3000 *avrB* [Shapiro and Zhang, 2001], so it is to be expected that the suppressor mutants would be much more susceptible to *Pst* DC3000 *avrRpt2* than to *Pst* DC3000 *avrB*. It has also been shown that RPT2, which confers resistance to Pst DC3000 (avrRpt2), and RPM1, which recognises the TTEs AvrB and AvrRpm1, associate with RIN4 in planta [Day et al., 2006]. It could be tested whether the association of RIN4 with RPS2 and RPM1 is impaired in *atgsnor1-3* and in the suppressor mutants. Furthermore, testing disease resistance of *atgsnor1-3 cat1*, *atgsnor1-3 cat2*, *atgsnor1-3 cat3*, and *atgsnor1-3 cat2 cat3* could provide useful insights. It is possible that a further increase of H_2O_2 levels in *atgsnor1-3 cat2* and *atgsnor1-3 cat2 cat3* would have a positive effect on SA-independent and ROS-dependent pathways and further increase disease resistance. However, since plant defence signalling is very finely tuned, it is also feasible that a loss of CAT2 or CAT2 and CAT3 results in too much H_2O_2 and has a detrimental effect on disease resistance.

It would also be useful to look at *PR1* expression and possibly other defence-related genes after challenge with various pathogens and SA. It appears that compared to *atgsnor1-3* and wild type, the suppressor mutants show intermediate *PR1* expression after *Pst* DC3000 (*avrRps4*) infection. It would be interesting to see if this is also observed after infection with other avirulent bacteria. Furthermore, it would be useful to look at *PR1* expression after challenge with virulent pathogens and after SA treatment. It can be speculated that infection with avirulent pathogens would lead to increased *PR1* expression due to activation of an SA-independent defence pathway, but that virulent pathogens and SA treatment are not able to induce *PR1* expression because the suppressor mutants are still impaired in SA signalling.

So far, only disease resistance to bacterial pathogens was tested and it would be advisable to also include other types as pathogens such as oomocytes. Furthermore, it is not clear if the suppressor mutants have recovered non-host resistance, so it would be useful to test if they are resistant to non host pathogens, such as *Pseudomonas syringae* pv *phaseolicola* or *Blumeria graminis* f. sp. *tritici*. It would also be interesting to see if SAR is compromised in the suppressor mutants.

It appears that partial recovery of disease resistance in the suppressor mutants might be due to a SA-independent signalling pathway. To identify components involved in this pathway, a microarray would be a good approach. This could potentially lead to the identification of previously unknown signalling pathways.

These results show that ROS and redox homeostasis play an important

role both in development and disease resistance, and that just a slight change
of ROS levels, caused by loss of CAT3 activity in the suppressor mutants, has
tremendous effects and can partially suppress the *atgsnor1-3* phenotype. It
would be interesting to see if these effects are limited to loss of CAT3 activity,
or if loss of other catalases or other redox-related genes also has an impact
on *atgsnor1-3*. To achieve this it would be necessary to fully characterise the
various double and triple mutants that were obtained, and look at their disease
resistance and HR. It would also be useful to measure SNO and ROI levels,
particularly in *atgnsor1-3 pad2*, *atgsnor1-3 trx3*, and *atgsnor1-3 trx5*. Loss-
of-function of various redox-related genes did not restore apical dominance
in *atgsnor1-3* but it is possible that it could result in increased or decreased
disease resistance or HR. *trx3* and *trx5* plants are impaired in SA-induced
PR1 induction and have lost SAR [Tada et al., 2008]. The *pad2-1* mutant
shows increased susceptibility to a wide range of pathogens and herbivorous
insects [Dubreuil-Maurizi et al., 2011]. Considering that loss of these genes
has a negative impact on disease resistance, it is perhaps unlikely that they
will be able to suppress the *atgsnor1-3* phenotype. However, loss of these
genes in an *atgsnor1-3* background might well lead to an even more severe
phenotype, which could also lead to interesting new insights into plant disease
resistance. *vtc2-1* on the other hand has been shown to be more resistant to
virulent pathogen, has higher SA levels and higher *PR1* expression [Colville
and Smirnoff, 2008, Barth et al., 2004, Pavet et al., 2005]. Unfortunately the
atgsnor1-3 vtc2-1 double mutant has not been obtained yet, but it could po-
tentially prove to be very interesting. It could also be interesting to overex-
press *CAT3* in *atgsnor1-3*, this could possibly lead to even more severe growth

phenotype and higher HR. *cat2* plants have been shown to have increased GSH levels [Han et al., 2013]. To test whether increased GSH levels play a role in suppressing the *atgsnor1-3* phenotype, it would be necessary to obtain a *atgsnor1-3 cat3 pad2* triple mutant. However, given that *atgsnor1-3 cat2*, which presumably has higher GSH levels than *atgsnor1-3 cat3*, has not recovered apical dominance, it is perhaps not very likely that increased GSH content in the supressor mutants plays a major role. Nevertheless, this triple mutant could still provide interesting, perhaps unexpected insights.

7.5   Conclusion

A suppressor screen uncovered two allelic suppressor mutations of *atgsnor1-3*, which were identified as mutations in *CAT3*. Catalase activity in the suppressor mutants was not significantly different from catalase activity in *cat3* plants, indicating that both mutations likely result in a complete loss of CAT3 activity. Interestingly, only loss of *CAT3*, but not loss of *CAT1* or *CAT2*, was able to recover apical dominance in the *atgsnor1-3* background, which is probably due to different temporal and/or spatial expression patterns of *CAT1*, *CAT2*, and *CAT3*. The suppressor mutants have recovered wild type HR, which is probably caused by a more balanced H_2O_2 to NO ratio compared to *atgsnor1-3*, but not by a change in SNO levels. The suppressor mutants are still susceptible to virulent pathogens, but have partially recovered disease resistance to avirulent pathogens. This phenotype is most likely caused by an SA-independent but ROS-dependent signalling pathway. However, because SA levels in the suppressor mutants remain low, the SA-dependent branch of plant

defence responses remains inactive, which explains why disease resistance was only partially restored.

Loss of CAT3 activity was able to partially suppress the *atgsnor1-3* phenotype, which is caused by excess SNO, thereby providing a link between S-nitrosylation and redox status. Interestingly, this suppression appears to be mainly due to a changed redox status in the suppressor mutants and not a change in SNO levels. Further characterisation of the suppressor mutants would be necessary to fully understand the mechanisms at work here, and this could possibly lead to the discovery of previously unknown defence signalling pathways.

References

[Aarts et al., 1998] Aarts, N., Metz, M., Holub, E., Staskawicz, B. J., Daniels, M. J. and Parker, J. E. (1998). Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in Arabidopsis. Proc Natl Acad Sci U S A *95*, 10306–10311.

[Abe et al., 2003] Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003). Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell *15*, 63–78.

[Abramovitch et al., 2006] Abramovitch, R. B., Anderson, J. C. and Martin, G. B. (2006). Bacterial elicitation and evasion of plant innate immunity. Nat Rev Mol Cell Biol *7*, 601–611.

[Abramovitch et al., 2003] Abramovitch, R. B., Kim, Y.-J., Chen, S., Dickman, M. B. and Martin, G. B. (2003). Pseudomonas type III effector AvrPtoB induces plant disease susceptibility by inhibition of host programmed cell death. EMBO J *22*, 60–69.

[Afzal et al., 2011] Afzal, A. J., da Cunha, L. and Mackey, D. (2011). Separable fragments and membrane tethering of Arabidopsis RIN4 regulate its suppression of PAMP-triggered immunity. Plant Cell *23*, 3798–3811.

[Akiyoshi et al., 1983] Akiyoshi, D. E., Morris, R. O., Hinz, R., Mischke, B. S., Kosuge, T., Garfinkel, D. J., Gordon, M. P. and Nester, E. W. (1983). Cytokinin/auxin balance in crown gall tumors is regulated by specific loci in the T-DNA. Proc Natl Acad Sci U S A *80*, 407–411.

[Alderton et al., 2001] Alderton, W. K., Cooper, C. E. and Knowles, R. G. (2001). Nitric oxide synthases: structure, function and inhibition. Biochem J *357*, 593–615.

[Alonso et al., 2003] Alonso, J. M., Stepanova, A. N., Leisse, T. J., Kim, C. J., Chen, H., Shinn, P., Stevenson, D. K., Zimmerman, J., Barajas, P., Cheuk, R., Gadrinab, C., Heller, C., Jeske, A., Koesema, E., Meyers, C. C., Parker, H., Prednis, L., Ansari, Y., Choy, N., Deen, H., Geralt, M., Hazari, N., Hom, E., Karnes, M., Mulholland, C., Ndubaku, R., Schmidt, I., Guzman, P., Aguilar-Henonin, L., Schmid, M., Weigel, D., Carter, D. E., Marchand, T., Risseeuw, E., Brogden, D., Zeko, A., Crosby, W. L., Berry, C. C. and Ecker, J. R. (2003). Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science *301*, 653–657.

[Anderson et al., 2004] Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., Maclean, D. J., Ebert, P. R. and Kazan, K. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell *16*, 3460–3479.

[Apel and Hirt, 2004] Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol *55*, 373–399.

[Arabidopsis Genome Initiative, 2000] Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature *408*, 796–815.

[Aravind and Koonin, 1999] Aravind, L. and Koonin, E. V. (1999). Fold prediction and evolutionary analysis of the POZ domain: structural and evolutionary relationship with the potassium channel tetramerization domain. J Mol Biol *285*, 1353–1361.

REFERENCES

[Asada, 2006] Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol *141*, 391–396.

[Asai et al., 2002] Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W.-L., Gomez-Gomez, L., Boller, T., Ausubel, F. M. and Sheen, J. (2002). MAP kinase signalling cascade in Arabidopsis innate immunity. Nature *415*, 977–983.

[Asselbergh et al., 2008] Asselbergh, B., De Vleesschauwer, D. and Höfte, M. (2008). Global switches and fine-tuning-ABA modulates plant pathogen defense. Mol Plant Microbe Interact *21*, 709–719.

[Astier et al., 2012] Astier, J., Kulik, A., Koen, E., Besson-Bard, A., Bourque, S., Jeandroz, S., Lamotte, O. and Wendehenne, D. (2012). Protein S-nitrosylation: what's going on in plants? Free Radic Biol Med *53*, 1101–1110.

[Attaran et al., 2009] Attaran, E., Zeier, T. E., Griebel, T. and Zeier, J. (2009). Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in Arabidopsis. The Plant Cell Online *21*, 954–971.

[Austin et al., 2002] Austin, M. J., Muskett, P., Kahn, K., Feys, B. J., Jones, J. D. and Parker, J. E. (2002). Regulatory role of SGT1 in early R gene-mediated plant defenses. Science *295*, 2077–2080.

[Axtell et al., 2003] Axtell, M. J., Chisholm, S. T., Dahlbeck, D. and Staskawicz, B. J. (2003). Genetic and molecular evidence that the Pseudomonas syringae type III effector protein AvrRpt2 is a cysteine protease. Mol Microbiol *49*, 1537–1546.

[Axtell and Staskawicz, 2003] Axtell, M. J. and Staskawicz, B. J. (2003). Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. Cell *112*, 369–377.

[Azevedo et al., 2002] Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K. and Schulze-Lefert, P. (2002). The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. Science *295*, 2073–2076.

[Ball et al., 2004] Ball, L., Accotto, G.-P., Bechtold, U., Creissen, G., Funck, D., Jimenez, A., Kular, B., Leyland, N., Mejia-Carranza, J., Reynolds, H., Karpinski, S. and Mullineaux, P. M. (2004). Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. Plant Cell *16*, 2448–2462.

[Ballaré, 2011] Ballaré, C. L. (2011). Jasmonate-induced defenses: a tale of intelligence, collaborators and rascals. Trends Plant Sci *16*, 249–257.

[Balmer et al., 2004] Balmer, Y., Vensel, W. H., Tanaka, C. K., Hurkman, W. J., Gelhaye, E., Rouhier, N., Jacquot, J.-P., Manieri, W., Schürmann, P., Droux, M. and Buchanan, B. B. (2004). Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria. Proc Natl Acad Sci U S A *101*, 2642–2647.

[Bardoel et al., 2011] Bardoel, B. W., van der Ent, S., Pel, M. J. C., Tommassen, J., Pieterse, C. M. J., van Kessel, K. P. M. and van Strijp, J. A. G. (2011). Pseudomonas evades immune recognition of flagellin in both mammals and plants. PLoS Pathog *7*, e1002206.

[Bari and Jones, 2009] Bari, R. and Jones, J. D. G. (2009). Role of plant hormones in plant defence responses. Plant Mol Biol *69*, 473–488.

[Barth et al., 2004] Barth, C., Moeder, W., Klessig, D. F. and Conklin, P. L. (2004). The timing of senescence and response to pathogens is altered in the ascorbate-deficient Arabidopsis mutant vitamin c-1. Plant Physiol *134*, 1784–1792.

[Bartsch et al., 2006] Bartsch, M., Gobbato, E., Bednarek, P., Debey, S., Schultze, J. L., Bautor, J. and Parker, J. E. (2006). Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in Arabidopsis immunity and cell death is regulated by the monooxygenase FMO1 and the Nudix hydrolase NUDT7. Plant Cell *18*, 1038–1051.

REFERENCES

[Bashandy et al., 2010] Bashandy, T., Guilleminot, J., Vernoux, T., Caparros-Ruiz, D., Ljung, K., Meyer, Y. and Reichheld, J.-P. (2010). Interplay between the NADP-linked thioredoxin and glutathione systems in Arabidopsis auxin signaling. Plant Cell *22*, 376–391.

[Bashandy et al., 2011] Bashandy, T., Meyer, Y. and Reichheld, J.-P. (2011). Redox regulation of auxin signaling and plant development in Arabidopsis. Plant Signal Behav *6*, 117–119.

[Baureithel et al., 1994] Baureithel, K., Felix, G. and Boller, T. (1994). Specific, high affinity binding of chitin fragments to tomato cells and membranes. Competitive inhibition of binding by derivatives of chitooligosaccharides and a Nod factor of Rhizobium. J Biol Chem *269*, 17931–17938.

[Bedini et al., 2005] Bedini, E., De Castro, C., Erbs, G., Mangoni, L., Dow, J. M., Newman, M.-A., Parrilli, M. and Unverzagt, C. (2005). Structure-dependent modulation of a pathogen response in plants by synthetic O-antigen polysaccharides. J Am Chem Soc *127*, 2414–2416.

[Belenghi et al., 2007] Belenghi, B., Romero-Puertas, M. C., Vercammen, D., Brackenier, A., Inzé, D., Delledonne, M. and Van Breusegem, F. (2007). Metacaspase activity of Arabidopsis thaliana is regulated by S-nitrosylation of a critical cysteine residue. Journal of Biological Chemistry *282*, 1352–1358.

[Belkhadir et al., 2004] Belkhadir, Y., Nimchuk, Z., Hubert, D. A., Mackey, D. and Dangl, J. L. (2004). Arabidopsis RIN4 negatively regulates disease resistance mediated by RPS2 and RPM1 downstream or independent of the NDR1 signal modulator and is not required for the virulence functions of bacterial type III effectors AvrRpt2 or AvrRpm1. Plant Cell *16*, 2822–2835.

[Benhar et al., 2009] Benhar, M., Forrester, M. T. and Stamler, J. S. (2009). Protein denitrosylation: enzymatic mechanisms and cellular functions. Nat Rev Mol Cell Biol *10*, 721–732.

[Benjamins and Scheres, 2008] Benjamins, R. and Scheres, B. (2008). Auxin: the looping star in plant development. Annu Rev Plant Biol *59*, 443–465.

[Benschop et al., 2007] Benschop, J. J., Mohammed, S., O'Flaherty, M., Heck, A. J. R., Slijper, M. and Menke, F. L. H. (2007). Quantitative phosphoproteomics of early elicitor signaling in Arabidopsis. Mol Cell Proteomics *6*, 1198–1214.

[Berrocal-Lobo et al., 2002] Berrocal-Lobo, M., Molina, A. and Solano, R. (2002). Constitutive expression of ETHYLENE-RESPONSE-FACTOR1 in Arabidopsis confers resistance to several necrotrophic fungi. Plant J *29*, 23–32.

[Besson-Bard et al., 2008] Besson-Bard, A., Pugin, A. and Wendehenne, D. (2008). New insights into nitric oxide signaling in plants. Annu Rev Plant Biol *59*, 21–39.

[Bethke et al., 2004] Bethke, P. C., Badger, M. R. and Jones, R. L. (2004). Apoplastic synthesis of nitric oxide by plant tissues. Plant Cell *16*, 332–341.

[Bhattacharjee et al., 2011] Bhattacharjee, S., Halane, M. K., Kim, S. H. and Gassmann, W. (2011). Pathogen effectors target Arabidopsis EDS1 and alter its interactions with immune regulators. Science *334*, 1405–1408.

[Bieri et al., 2004] Bieri, S., Mauch, S., Shen, Q.-H., Peart, J., Devoto, A., Casais, C., Ceron, F., Schulze, S., Steinbiss, H.-H., Shirasu, K. and Schulze-Lefert, P. (2004). RAR1 positively controls steady state levels of barley MLA resistance proteins and enables sufficient MLA6 accumulation for effective resistance. Plant Cell *16*, 3480–3495.

[Bisgrove et al., 1994] Bisgrove, S. R., Simonich, M. T., Smith, N. M., Sattler, A. and Innes, R. W. (1994). A disease resistance gene in Arabidopsis with specificity for two different pathogen avirulence genes. Plant Cell *6*, 927–933.

[Boller, 1995] Boller, T. (1995). Chemoperception of microbial signals in plant cells. Annual review of plant biology *46*, 189–214.

REFERENCES

[Boller and Felix, 2009] Boller, T. and Felix, G. (2009). A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol *60*, 379–406.

[Bonas and Lahaye, 2002] Bonas, U. and Lahaye, T. (2002). Plant disease resistance triggered by pathogen-derived molecules: refined models of specific recognition. Curr Opin Microbiol *5*, 44–50.

[Bostock, 2005] Bostock, R. M. (2005). Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu Rev Phytopathol *43*, 545–580.

[Boyle et al., 2009] Boyle, P., Le Su, E., Rochon, A., Shearer, H. L., Murmu, J., Chu, J. Y., Fobert, P. R. and Després, C. (2009). The BTB/POZ domain of the Arabidopsis disease resistance protein NPR1 interacts with the repression domain of TGA2 to negate its function. Plant Cell *21*, 3700–3713.

[Bretz et al., 2003] Bretz, J. R., Mock, N. M., Charity, J. C., Zeyad, S., Baker, C. J. and Hutcheson, S. W. (2003). A translocated protein tyrosine phosphatase of Pseudomonas syringae pv. tomato DC3000 modulates plant defence response to infection. Mol Microbiol *49*, 389–400.

[Brodersen et al., 2006] Brodersen, P., Petersen, M., Bjørn Nielsen, H., Zhu, S., Newman, M.-A., Shokat, K. M., Rietz, S., Parker, J. and Mundy, J. (2006). Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD4. Plant J *47*, 532–546.

[Broekaert et al., 2006] Broekaert, W. F., Delauré, S. L., De Bolle, M. F. C. and Cammue, B. P. A. (2006). The role of ethylene in host-pathogen interactions. Annu Rev Phytopathol *44*, 393–416.

[Brooks et al., 2005] Brooks, D. M., Bender, C. L. and Kunkel, B. N. (2005). The Pseudomonas syringae phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in Arabidopsis thaliana. Mol Plant Pathol *6*, 629–639.

[Browse, 2009] Browse, J. (2009). Jasmonate passes muster: a receptor and targets for the defense hormone. Annu Rev Plant Biol *60*, 183–205.

[Bueso et al., 2007] Bueso, E., Alejandro, S., Carbonell, P., Perez-Amador, M. A., Fayos, J., Bellés, J. M., Rodriguez, P. L. and Serrano, R. (2007). The lithium tolerance of the Arabidopsis cat2 mutant reveals a cross-talk between oxidative stress and ethylene. Plant J *52*, 1052–1065.

[Cao et al., 2011] Cao, F. Y., Yoshioka, K. and Desveaux, D. (2011). The roles of ABA in plant-pathogen interactions. J Plant Res *124*, 489–499.

[Cao et al., 1997] 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.

[Century et al., 1995] Century, K. S., Holub, E. B. and Staskawicz, B. J. (1995). NDR1, a locus of Arabidopsis thaliana that is required for disease resistance to both a bacterial and a fungal pathogen. Proc Natl Acad Sci U S A *92*, 6597–6601.

[Century et al., 1997] Century, K. S., Shapiro, A. D., Repetti, P. P., Dahlbeck, D., Holub, E. and Staskawicz, B. J. (1997). NDR1, a pathogen-induced component required for Arabidopsis disease resistance. Science *278*, 1963–1965.

[Chandra et al., 1997] Chandra, S., Stennis, M. and Low, P. S. (1997). Measurement of Ca2+ fluxes during elicitation of the oxidative burst in aequorin-transformed tobacco cells. J Biol Chem *272*, 28274–28280.

[Chandra-Shekara et al., 2004] Chandra-Shekara, A. C., Navarre, D., Kachroo, A., Kang, H.-G., Klessig, D. and Kachroo, P. (2004). Signaling requirements and role of salicylic acid in HRT- and rrt-mediated resistance to turnip crinkle virus in Arabidopsis. Plant J *40*, 647–659.

REFERENCES

[Chaouch et al., 2012] Chaouch, S., Queval, G. and Noctor, G. (2012). AtRbohF is a crucial modulator of defence-associated metabolism and a key actor in the interplay between intracellular oxidative stress and pathogenesis responses in Arabidopsis. Plant J *69*, 613–627.

[Chaouch et al., 2010] Chaouch, S., Queval, G., Vanderauwera, S., Mhamdi, A., Vandorpe, M., Langlois-Meurinne, M., Van Breusegem, F., Saindrenan, P. and Noctor, G. (2010). Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORIS-MATE SYNTHASE1 in a daylength-related manner. Plant Physiol *153*, 1692–1705.

[Chen et al., 2009] Chen, H., Xue, L., Chintamanani, S., Germain, H., Lin, H., Cui, H., Cai, R., Zuo, J., Tang, X., Li, X., Guo, H. and Zhou, J.-M. (2009). ETHYLENE IN-SENSITIVE3 and ETHYLENE INSENSITIVE3-LIKE1 repress SALICYLIC ACID IN-DUCTION DEFICIENT2 expression to negatively regulate plant innate immunity in Arabidopsis. Plant Cell *21*, 2527–2540.

[Chen et al., 2010] Chen, L., Zhang, L. and Yu, D. (2010). Wounding-induced WRKY8 is involved in basal defense in Arabidopsis. Mol Plant Microbe Interact *23*, 558–565.

[Chen et al., 2009] Chen, R., Sun, S., Wang, C., Li, Y., Liang, Y., An, F., Li, C., Dong, H., Yang, X., Zhang, J. and Zuo, J. (2009). The Arabidopsis PARAQUAT RESISTANT2 gene encodes an S-nitrosoglutathione reductase that is a key regulator of cell death. Cell Res *19*, 1377–1387.

[Chen et al., 2007] Chen, Z., Agnew, J. L., Cohen, J. D., He, P., Shan, L., Sheen, J. and Kunkel, B. N. (2007). Pseudomonas syringae type III effector AvrRpt2 alters Arabidopsis thaliana auxin physiology. Proc Natl Acad Sci U S A *104*, 20131–20136.

[Chen et al., 2004] Chen, Z., Kloek, A. P., Cuzick, A., Moeder, W., Tang, D., Innes, R. W., Klessig, D. F., McDowell, J. M. and Kunkel, B. N. (2004). The Pseudomonas syringae type III effector AvrRpt2 functions downstream or independently of SA to promote virulence on Arabidopsis thaliana. Plant J *37*, 494–504.

[Chen et al., 1993] Chen, Z., Silva, H. and Klessig, D. F. (1993). Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science *262*, 1883–1886.

[Cheng et al., 2009] Cheng, Y. T., Germain, H., Wiermer, M., Bi, D., Xu, F., García, A. V., Wirthmueller, L., Després, C., Parker, J. E., Zhang, Y. and Li, X. (2009). Nuclear pore complex component MOS7/Nup88 is required for innate immunity and nuclear accumulation of defense regulators in Arabidopsis. Plant Cell *21*, 2503–2516.

[Chinchilla et al., 2007] Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nürnberger, T., Jones, J. D. G., Felix, G. and Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature *448*, 497–500.

[Chini et al., 2007] Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J. M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F. M., Ponce, M. R., Micol, J. L. and Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature *448*, 666–671.

[Chisholm et al., 2006] Chisholm, S. T., Coaker, G., Day, B. and Staskawicz, B. J. (2006). Host-microbe interactions: shaping the evolution of the plant immune response. Cell *124*, 803–814.

[Choi et al., 2011] Choi, J., Choi, D., Lee, S., Ryu, C.-M. and Hwang, I. (2011). Cytokinins and plant immunity: old foes or new friends? Trends Plant Sci *16*, 388–394.

[Chung et al., 2011] Chung, E.-H., da Cunha, L., Wu, A.-J., Gao, Z., Cherkis, K., Afzal, A. J., Mackey, D. and Dangl, J. L. (2011). Specific threonine phosphorylation of a host target by two unrelated type III effectors activates a host innate immune receptor in plants. Cell Host Microbe *9*, 125–136.

[Chung et al., 2009] Chung, H. S., Niu, Y., Browse, J. and Howe, G. A. (2009). Top hits in contemporary JAZ: an update on jasmonate signaling. Phytochemistry *70*, 1547–1559.

REFERENCES

[Clarke et al., 2009] Clarke, S. M., Cristescu, S. M., Miersch, O., Harren, F. J. M., Waster-nack, C. and Mur, L. A. J. (2009). Jasmonates act with salicylic acid to confer basal thermotolerance in Arabidopsis thaliana. New Phytol *182*, 175–187.

[Clough and Bent, 1998] Clough, S. J. and Bent, A. F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J *16*, 735–743.

[Clough et al., 2000] Clough, S. J., Fengler, K. A., Yu, I. C., Lippok, B., Smith, Jr, R. 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.

[Coaker et al., 2005] Coaker, G., Falick, A. and Staskawicz, B. (2005). Activation of a phytopathogenic bacterial effector protein by a eukaryotic cyclophilin. Science *308*, 548–550.

[Colcombet and Hirt, 2008] Colcombet, J. and Hirt, H. (2008). Arabidopsis MAPKs: a complex signalling network involved in multiple biological processes. Biochem J *413*, 217–226.

[Collmer et al., 2002] Collmer, A., Lindeberg, M., Petnicki-Ocwieja, T., Schneider, D. J. and Alfano, J. R. (2002). Genomic mining type III secretion system effectors in Pseudomonas syringae yields new picks for all TTSS prospectors. Trends Microbiol *10*, 462–469.

[Colville and Smirnoff, 2008] Colville, L. and Smirnoff, N. (2008). Antioxidant status, peroxidase activity, and PR protein transcript levels in ascorbate-deficient Arabidopsis thaliana vtc mutants. J Exp Bot *59*, 3857–3868.

[Conklin et al., 2000] Conklin, P. L., Saracco, S. A., Norris, S. R. and Last, R. L. (2000). Identification of ascorbic acid-deficient Arabidopsis thaliana mutants. Genetics *154*, 847–856.

[Contento and Bassham, 2010] Contento, A. L. and Bassham, D. C. (2010). Increase in catalase-3 activity as a response to use of alternative catabolic substrates during sucrose starvation. Plant Physiol Biochem *48*, 232–238.

[Coppinger et al., 2004] Coppinger, P., Repetti, P. P., Day, B., Dahlbeck, D., Mehlert, A. and Staskawicz, B. J. (2004). Overexpression of the plasma membrane-localized NDR1 protein results in enhanced bacterial disease resistance in Arabidopsis thaliana. Plant J *40*, 225–237.

[Corpas et al., 2006] Corpas, F. J., Barroso, J. B., Carreras, A., Valderrama, R., Palma, J. M., León, A. M., Sandalio, L. M. and del Río, L. A. (2006). Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta *224*, 246–254.

[Corpas et al., 2008] Corpas, F. J., Chaki, M., Fernández-Ocaña, A., Valderrama, R., Palma, J. M., Carreras, A., Begara-Morales, J. C., Airaki, M., del Río, L. A. and Barroso, J. B. (2008). Metabolism of reactive nitrogen species in pea plants under abiotic stress conditions. Plant Cell Physiol *49*, 1711–1722.

[Correa-Aragunde et al., 2004] Correa-Aragunde, N., Graziano, M. and Lamattina, L. (2004). Nitric oxide plays a central role in determining lateral root development in tomato. Planta *218*, 900–905.

[Costa et al., 2010] Costa, A., Drago, I., Behera, S., Zottini, M., Pizzo, P., Schroeder, J. I., Pozzan, T. and Lo Schiavo, F. (2010). H2O2 in plant peroxisomes: an in vivo analysis uncovers a Ca(2+)-dependent scavenging system. Plant J *62*, 760–772.

[Costa et al., 2013] Costa, A., Drago, I., Zottini, M., Pizzo, P. and Pozzan, T. (2013). Peroxisome ca(2+) homeostasis in animal and plant cells. Subcell Biochem *69*, 111–133.

[Costacurta and Vanderleyden, 1995] Costacurta, A. and Vanderleyden, J. (1995). Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol *21*, 1–18.

[Crawford et al., 2006] Crawford, N. M., Galli, M., Tischner, R., Heimer, Y. M., Okamoto, M. and Mack, A. (2006). Response to Zemojtel et al: Plant nitric oxide synthase: back to square one. Trends in Plant Science *11*, 526–527.

REFERENCES

[Dai et al., 2006] Dai, Y., Wang, H., Li, B., Huang, J., Liu, X., Zhou, Y., Mou, Z. and Li, J. (2006). Increased expression of MAP KINASE KINASE7 causes deficiency in polar auxin transport and leads to plant architectural abnormality in Arabidopsis. Plant Cell *18*, 308–320.

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

[Dat et al., 2000] Dat, J., Vandenabeele, S., Vranová, E., Van Montagu, M., Inzé, D. and Van Breusegem, F. (2000). Dual action of the active oxygen species during plant stress responses. Cell Mol Life Sci *57*, 779–795.

[Daudi et al., 2012] Daudi, A., Cheng, Z., O'Brien, J. A., Mammarella, N., Khan, S., Ausubel, F. M. and Bolwell, G. P. (2012). The apoplastic oxidative burst peroxidase in Arabidopsis is a major component of pattern-triggered immunity. Plant Cell *24*, 275–287.

[Davletova et al., 2005] Davletova, S., Rizhsky, L., Liang, H., Shengqiang, Z., Oliver, D. J., Coutu, J., Shulaev, V., Schlauch, K. and Mittler, R. (2005). Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. Plant Cell *17*, 268–281.

[Day et al., 2005] Day, B., Dahlbeck, D., Huang, J., Chisholm, S. T., Li, D. and Staskawicz, B. J. (2005). Molecular basis for the RIN4 negative regulation of RPS2 disease resistance. Plant Cell *17*, 1292–1305.

[Day et al., 2006] Day, B., Dahlbeck, D. and Staskawicz, B. J. (2006). NDR1 interaction with RIN4 mediates the differential activation of multiple disease resistance pathways in Arabidopsis. Plant Cell *18*, 2782–2791.

[Day et al., 2001] Day, R. B., Okada, M., Ito, Y., Tsukada, K., Zaghouani, H., Shibuya, N. and Stacey, G. (2001). Binding site for chitin oligosaccharides in the soybean plasma membrane. Plant Physiol *126*, 1162–1173.

[Díaz et al., 2003] Díaz, M., Achkor, H., Titarenko, E. and Martínez, M. C. (2003). The gene encoding glutathione-dependent formaldehyde dehydrogenase/GSNO reductase is responsive to wounding, jasmonic acid and salicylic acid. FEBS Lett *543*, 136–139.

[de Jonge et al., 2010] de Jonge, R., van Esse, H. P., Kombrink, A., Shinya, T., Desaki, Y., Bours, R., van der Krol, S., Shibuya, N., Joosten, M. H. A. J. and Thomma, B. P. H. J. (2010). Conserved fungal LysM effector Ecp6 prevents chitin-triggered immunity in plants. Science *329*, 953–955.

[de la Fuente van Bentem and Hirt, 2007] de la Fuente van Bentem, S. and Hirt, H. (2007). Using phosphoproteomics to reveal signalling dynamics in plants. Trends Plant Sci *12*, 404–411.

[de Torres Zabala et al., 2009] de Torres Zabala, M., Bennett, M. H., Truman, W. H. and Grant, M. R. (2009). Antagonism between salicylic and abscisic acid reflects early host-pathogen conflict and moulds plant defence responses. Plant J *59*, 375–386.

[de Torres-Zabala et al., 2007] de Torres-Zabala, M., Truman, W., Bennett, M. H., Lafforgue, G., Mansfield, J. W., Rodriguez Egea, P., Bögre, L. and Grant, M. (2007). Pseudomonas syringae pv. tomato hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. EMBO J *26*, 1434–1443.

[De Vos et al., 2006] De Vos, M., Van Zaanen, W., Koornneef, A., Korzelius, J. P., Dicke, M., Van Loon, L. C. and Pieterse, C. M. J. (2006). Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiol *142*, 352–363.

[DebRoy et al., 2004] DebRoy, S., Thilmony, R., Kwack, Y.-B., Nomura, K. and He, S. Y. (2004). A family of conserved bacterial effectors inhibits salicylic acid-mediated basal immunity and promotes disease necrosis in plants. Proc Natl Acad Sci U S A *101*, 9927–9932.

[del Río et al., 2006] del Río, L. A., Sandalio, L. M., Corpas, F. J., Palma, J. M. and Barroso, J. B. (2006). Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol *141*, 330–335.

[Delaney, 1997] Delaney, T. P. (1997). Genetic dissection of acquired resistance to disease. Plant Physiol *113*, 5–12.

[DellaPenna and Pogson, 2006] DellaPenna, D. and Pogson, B. J. (2006). Vitamin synthesis in plants: tocopherols and carotenoids. Annu Rev Plant Biol *57*, 711–738.

[Delledonne, 2005] Delledonne, M. (2005). NO news is good news for plants. Curr Opin Plant Biol *8*, 390–396.

[Delledonne et al., 1998] 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 et al., 2001] 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.

[Dempsey et al., 1999] Dempsey, D. A., Shah, J. and Klessig, D. F. (1999). Salicylic acid and disease resistance in plants. Critical Reviews in Plant Sciences *18*, 547–575.

[Desikan et al., 2001] Desikan, R., A-H-Mackerness, S., Hancock, J. T. and Neill, S. J. (2001). Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol *127*, 159–172.

[Deslandes et al., 2003] Deslandes, L., Olivier, J., Peeters, N., Feng, D. X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S. and Marco, Y. (2003). Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc Natl Acad Sci U S A *100*, 8024–8029.

[Deslandes and Rivas, 2012] Deslandes, L. and Rivas, S. (2012). Catch me if you can: bacterial effectors and plant targets. Trends Plant Sci *17*, 644–655.

[Després et al., 2003] Després, C., Chubak, C., Rochon, A., Clark, R., Bethune, T., Desveaux, D. and Fobert, P. R. (2003). The Arabidopsis NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell *15*, 2181–2191.

[Després et al., 2000] Després, 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.

[Desveaux et al., 2004] Desveaux, D., Subramaniam, R., Després, C., Mess, J.-N., Lévesque, C., Fobert, P. R., Dangl, J. L. and Brisson, N. (2004). A "Whirly" transcription factor is required for salicylic acid-dependent disease resistance in Arabidopsis. Dev Cell *6*, 229–240.

[Dietz, 2003] Dietz, K.-J. (2003). Plant peroxiredoxins. Annu Rev Plant Biol *54*, 93–107.

[Diezel et al., 2009] Diezel, C., von Dahl, C. C., Gaquerel, E. and Baldwin, I. T. (2009). Different lepidopteran elicitors account for cross-talk in herbivory-induced phytohormone signaling. Plant Physiol *150*, 1576–1586.

[Dodds et al., 2006] Dodds, P. N., Lawrence, G. J., Catanzariti, A.-M., Teh, T., Wang, C.-I. A., Ayliffe, M. A., Kobe, B. and Ellis, J. G. (2006). Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. Proc Natl Acad Sci U S A *103*, 8888–8893.

[Dombrecht et al., 2007] Dombrecht, B., Xue, G. P., Sprague, S. J., Kirkegaard, J. A., Ross, J. J., Reid, J. B., Fitt, G. P., Sewelam, N., Schenk, P. M., Manners, J. M. and Kazan, K. (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in Arabidopsis. Plant Cell *19*, 2225–2245.

[Dong et al., 2003] Dong, J., Chen, C. and Chen, Z. (2003). Expression profiles of the Arabidopsis WRKY gene superfamily during plant defense response. Plant Mol Biol *51*, 21–37.

REFERENCES

[Dong, 2004] Dong, X. (2004). NPR1, all things considered. Curr Opin Plant Biol *7*, 547–552.

[Dordas et al., 2004] Dordas, C., Hasinoff, B. B., Rivoal, J. and Hill, R. D. (2004). Class-1 hemoglobins, nitrate and NO levels in anoxic maize cell-suspension cultures. Planta *219*, 66–72.

[Dorey et al., 1998] Dorey, S., Baillieul, F., Saindrenan, P., Fritig, B. and Kauffmann, S. (1998). Tobacco class I and II catalases are differentially expressed during elicitor-induced hypersensitive cell death and localized acquired resistance. Molecular plant-microbe interactions *11*, 1102–1109.

[Dorffling et al., 1984] Dorffling, K., Petersen, W., Sprecher, E., Urbasch, I. and Hanssen, H.-P. (1984). Abscisic acid in phytopathogenic fungi of the genera Botrytis, Ceratocystis, Fusarium, and Rhizoctonia. Zeitschrift fur Naturforschung. Section C, Biosciences *39*, 683–684.

[Dowdle et al., 2007] Dowdle, J., Ishikawa, T., Gatzek, S., Rolinski, S. and Smirnoff, N. (2007). Two genes in Arabidopsis thaliana encoding GDP-L-galactose phosphorylase are required for ascorbate biosynthesis and seedling viability. Plant J *52*, 673–689.

[Drenkard et al., 2000] Drenkard, E., Richter, B. G., Rozen, S., Stutius, L. M., Angell, N. A., Mindrinos, M., Cho, R. J., Oefner, P. J., Davis, R. W. and Ausubel, F. M. (2000). A simple procedure for the analysis of single nucleotide polymorphisms facilitates map-based cloning in Arabidopsis. Plant Physiol *124*, 1483–1492.

[Du and Klessig, 1997] Du, H. and Klessig, D. F. (1997). Identification of a Soluble, High-Affinity Salicylic Acid-Binding Protein in Tobacco. Plant Physiol *113*, 1319–1327.

[Du et al., 2009] Du, L., Ali, G. S., Simons, K. A., Hou, J., Yang, T., Reddy, A. S. N. and Poovaiah, B. W. (2009). Ca(2+)/calmodulin regulates salicylic-acid-mediated plant immunity. Nature *457*, 1154–1158.

[Du et al., 2008] Du, Y.-Y., Wang, P.-C., Chen, J. and Song, C.-P. (2008). Comprehensive functional analysis of the catalase gene family in Arabidopsis thaliana. J Integr Plant Biol *50*, 1318–1326.

[Dubreuil-Maurizi et al., 2011] Dubreuil-Maurizi, C., Vitecek, J., Marty, L., Branciard, L., Frettinger, P., Wendehenne, D., Meyer, A. J., Mauch, F. and Poinssot, B. (2011). Glutathione deficiency of the Arabidopsis mutant pad2-1 affects oxidative stress-related events, defense gene expression, and the hypersensitive response. Plant Physiol *157*, 2000–2012.

[Durner and Klessig, 1999] Durner, J. and Klessig, D. F. (1999). Nitric oxide as a signal in plants. Curr Opin Plant Biol *2*, 369–374.

[Durner et al., 1997] Durner, J., Shah, J. and Klessig, D. F. (1997). Salicylic acid and disease resistance in plants. Trends in Plant Science *2*, 266–274.

[Durner et al., 1998] 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.

[Durrant and Dong, 2004] Durrant, W. E. and Dong, X. (2004). Systemic acquired resistance. Annu Rev Phytopathol *42*, 185–209.

[Durrant et al., 2000] Durrant, W. E., Rowland, O., Piedras, P., Hammond-Kosack, K. E. and Jones, J. D. (2000). cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles. Plant Cell *12*, 963–977.

[Durrant et al., 2007] Durrant, W. E., Wang, S. and Dong, X. (2007). Arabidopsis SNI1 and RAD51D regulate both gene transcription and DNA recombination during the defense response. Proc Natl Acad Sci U S A *104*, 4223–4227.

[Epple et al., 2003] Epple, P., Mack, A. A., Morris, V. R. F. and Dangl, J. L. (2003). Antagonistic control of oxidative stress-induced cell death in Arabidopsis by two related, plant-specific zinc finger proteins. Proc Natl Acad Sci U S A *100*, 6831–6836.

REFERENCES

[Espinosa et al., 2003] Espinosa, A., Guo, M., Tam, V. C., Fu, Z. Q. and Alfano, J. R. (2003). The Pseudomonas syringae type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase activity and suppresses programmed cell death in plants. Mol Microbiol *49*, 377–387.

[Espunya et al., 2012] Espunya, M. C., De Michele, R., Gómez-Cadenas, A. and Martínez, M. C. (2012). S-Nitrosoglutathione is a component of wound- and salicylic acid-induced systemic responses in Arabidopsis thaliana. J Exp Bot *63*, 3219–3227.

[Eulgem, 2005] Eulgem, T. (2005). Regulation of the Arabidopsis defense transcriptome. Trends Plant Sci *10*, 71–78.

[Eulgem and Somssich, 2007] Eulgem, T. and Somssich, I. E. (2007). Networks of WRKY transcription factors in defense signaling. Curr Opin Plant Biol *10*, 366–371.

[Falk et al., 1999] Falk, A., Feys, B. J., Frost, L. N., Jones, J. D., Daniels, M. J. and Parker, J. E. (1999). EDS1, an essential component of R gene-mediated disease resistance in Arabidopsis has homology to eukaryotic lipases. Proc Natl Acad Sci U S A *96*, 3292–3297.

[Fan and Dong, 2002] Fan, W. and Dong, X. (2002). In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in Arabidopsis. Plant Cell *14*, 1377–1389.

[Feechan et al., 2005] Feechan, A., Kwon, E., Yun, B.-W., Wang, Y., Pallas, J. A. and Loake, G. J. (2005). A central role for S-nitrosothiols in plant disease resistance. Proc Natl Acad Sci U S A *102*, 8054–8059.

[Felix et al., 1999] Felix, G., Duran, J. D., Volko, S. and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J *18*, 265–276.

[Felton and Korth, 2000] Felton, G. W. and Korth, K. L. (2000). Trade-offs between pathogen and herbivore resistance. Curr Opin Plant Biol *3*, 309–314.

[Felton and Tumlinson, 2008] Felton, G. W. and Tumlinson, J. H. (2008). Plant-insect dialogs: complex interactions at the plant-insect interface. Curr Opin Plant Biol *11*, 457–463.

[Fernández-Calvo et al., 2011] Fernández-Calvo, P., Chini, A., Fernández-Barbero, G., Chico, J.-M., Gimenez-Ibanez, S., Geerinck, J., Eeckhout, D., Schweizer, F., Godoy, M., Franco-Zorrilla, J. M., Pauwels, L., Witters, E., Puga, M. I., Paz-Ares, J., Goossens, A., Reymond, P., De Jaeger, G. and Solano, R. (2011). The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. Plant Cell *23*, 701–715.

[Fernández-Marcos et al., 2011] Fernández-Marcos, M., Sanz, L., Lewis, D. R., Muday, G. K. and Lorenzo, O. (2011). Nitric oxide causes root apical meristem defects and growth inhibition while reducing PIN-FORMED 1 (PIN1)-dependent acropetal auxin transport. Proc Natl Acad Sci U S A *108*, 18506–18511.

[Feys et al., 2001] Feys, B. J., Moisan, L. J., Newman, M. A. and Parker, J. E. (2001). Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4. EMBO J *20*, 5400–5411.

[Feys et al., 2005] Feys, B. J., Wiermer, M., Bhat, R. A., Moisan, L. J., Medina-Escobar, N., Neu, C., Cabral, A. and Parker, J. E. (2005). Arabidopsis SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. Plant Cell *17*, 2601–2613.

[Fonseca et al., 2009a] Fonseca, S., Chico, J. M. and Solano, R. (2009a). The jasmonate pathway: the ligand, the receptor and the core signalling module. Curr Opin Plant Biol *12*, 539–547.

[Fonseca et al., 2009b] Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, C. and Solano, R. (2009b). (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. Nat Chem Biol *5*, 344–350.

REFERENCES

[Foreman et al., 2003] Foreman, J., Demidchik, V., Bothwell, J. H. F., Mylona, P., Miedema, H., Torres, M. A., Linstead, P., Costa, S., Brownlee, C., Jones, J. D. G., Davies, J. M. and Dolan, L. (2003). Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature *422*, 442–446.

[Foresi et al., 2010] Foresi, N., Correa-Aragunde, N., Parisi, G., Caló, G., Salerno, G. and Lamattina, L. (2010). Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga Ostreococcus tauri is light irradiance and growth phase dependent. Plant Cell *22*, 3816–3830.

[Forouhar et al., 2005] Forouhar, F., Yang, Y., Kumar, D., Chen, Y., Fridman, E., Park, S. W., Chiang, Y., Acton, T. B., Montelione, G. T., Pichersky, E., Klessig, D. F. and Tong, L. (2005). Structural and biochemical studies identify tobacco SABP2 as a methyl salicylate esterase and implicate it in plant innate immunity. Proc Natl Acad Sci U S A *102*, 1773–1778.

[Foster et al., 2009] Foster, M. W., Liu, L., Zeng, M., Hess, D. T. and Stamler, J. S. (2009). A genetic analysis of nitrosative stress. Biochemistry *48*, 792–799.

[Foster et al., 2003] Foster, M. W., McMahon, T. J. and Stamler, J. S. (2003). S-nitrosylation in health and disease. Trends Mol Med *9*, 160–168.

[Foyer and Noctor, 2011] Foyer, C. H. and Noctor, G. (2011). Ascorbate and glutathione: the heart of the redox hub. Plant Physiol *155*, 2–18.

[Frugoli et al., 1996] Frugoli, J. A., Zhong, H. H., Nuccio, M. L., McCourt, P., McPeek, M. A., Thomas, T. L. and McClung, C. R. (1996). Catalase is encoded by a multigene family in Arabidopsis thaliana (L.) Heynh. Plant Physiol *112*, 327–336.

[Frye et al., 2001] Frye, C. A., Tang, D. and Innes, R. W. (2001). Negative regulation of defense responses in plants by a conserved MAPKK kinase. Proc Natl Acad Sci U S A *98*, 373–378.

[Fu et al., 2012] Fu, Z. Q., Yan, S., Saleh, A., Wang, W., Ruble, J., Oka, N., Mohan, R., Spoel, S. H., Tada, Y., Zheng, N. and Dong, X. (2012). NPR3 and NPR4 are receptors for the immune signal salicylic acid in plants. Nature *486*, 228–232.

[Fukamatsu et al., 2003] Fukamatsu, Y., Yabe, N. and Hasunuma, K. (2003). Arabidopsis NDK1 is a component of ROS signaling by interacting with three catalases. Plant Cell Physiol *44*, 982–989.

[Gadjev et al., 2008] Gadjev, I., Stone, J. M. and Gechev, T. S. (2008). Programmed cell death in plants: new insights into redox regulation and the role of hydrogen peroxide. Int Rev Cell Mol Biol *270*, 87–144.

[Gao et al., 2011] Gao, Q.-M., Venugopal, S., Navarre, D. and Kachroo, A. (2011). Low oleic acid-derived repression of jasmonic acid-inducible defense responses requires the WRKY50 and WRKY51 proteins. Plant Physiol *155*, 464–476.

[Gapper and Dolan, 2006] Gapper, C. and Dolan, L. (2006). Control of plant development by reactive oxygen species. Plant Physiol *141*, 341–345.

[García et al., 2010] García, A. V., Blanvillain-Baufumé, S., Huibers, R. P., Wiermer, M., Li, G., Gobbato, E., Rietz, S. and Parker, J. E. (2010). Balanced nuclear and cytoplasmic activities of EDS1 are required for a complete plant innate immune response. PLoS Pathog *6*, e1000970.

[Gassmann et al., 1999] Gassmann, W., Hinsch, M. E. and Staskawicz, B. J. (1999). The Arabidopsis RPS4 bacterial-resistance gene is a member of the TIR-NBS-LRR family of disease-resistance genes. Plant J *20*, 265–277.

[Gechev and Hille, 2005] Gechev, T. S. and Hille, J. (2005). Hydrogen peroxide as a signal controlling plant programmed cell death. J Cell Biol *168*, 17–20.

[Gechev et al., 2006] Gechev, T. S., Van Breusegem, F., Stone, J. M., Denev, I. and Laloi, C. (2006). Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. Bioessays *28*, 1091–1101.

REFERENCES

[Gfeller et al., 2010] Gfeller, A., Dubugnon, L., Liechti, R. and Farmer, E. E. (2010). Jasmonate biochemical pathway. Sci Signal *3*, cm3.

[Gil et al., 2001] Gil, P., Dewey, E., Friml, J., Zhao, Y., Snowden, K. C., Putterill, J., Palme, K., Estelle, M. and Chory, J. (2001). BIG: a calossin-like protein required for polar auxin transport in Arabidopsis. Genes Dev *15*, 1985–1997.

[Glazebrook, 2005] Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol *43*, 205–227.

[Glazebrook et al., 2003] Glazebrook, J., Chen, W., Estes, B., Chang, H.-S., Nawrath, C., Métraux, J.-P., Zhu, T. and Katagiri, F. (2003). Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. Plant J *34*, 217–228.

[Gälweiler et al., 1998] Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A. and Palme, K. (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science *282*, 2226–2230.

[Gómez-Gómez and Boller, 2000] Gómez-Gómez, L. and Boller, T. (2000). FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. Mol Cell *5*, 1003–1011.

[Gómez-Gómez and Boller, 2002] Gómez-Gómez, L. and Boller, T. (2002). Flagellin perception: a paradigm for innate immunity. Trends Plant Sci *7*, 251–256.

[Gómez-Gómez et al., 1999] Gómez-Gómez, L., Felix, G. and Boller, T. (1999). A single locus determines sensitivity to bacterial flagellin in Arabidopsis thaliana. Plant J *18*, 277–284.

[Godiard et al., 2003] Godiard, L., Sauviac, L., Torii, K. U., Grenon, O., Mangin, B., Grimsley, N. H. and Marco, Y. (2003). ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. Plant J *36*, 353–365.

[Granado et al., 1995] Granado, J., Felix, G. and Boller, T. (1995). Perception of Fungal Sterols in Plants (Subnanomolar Concentrations of Ergosterol Elicit Extracellular Alkalinization in Tomato Cells). Plant Physiol *107*, 485–490.

[Grant and Loake, 2000] Grant, J. J. and Loake, G. J. (2000). Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. Plant Physiol *124*, 21–29.

[Grant et al., 2000] Grant, M., Brown, I., Adams, S., Knight, M., Ainslie, A. and Mansfield, J. (2000). The RPM1 plant disease resistance gene facilitates a rapid and sustained increase in cytosolic calcium that is necessary for the oxidative burst and hypersensitive cell death. Plant J *23*, 441–450.

[Grant et al., 1995] Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R. W. and Dangl, J. L. (1995). Structure of the Arabidopsis RPM1 gene enabling dual specificity disease resistance. Science *269*, 843–846.

[Grant and Jones, 2009] Grant, M. R. and Jones, J. D. G. (2009). Hormone (dis)harmony moulds plant health and disease. Science *324*, 750–752.

[Greene et al., 2003] Greene, E. A., Codomo, C. A., Taylor, N. E., Henikoff, J. G., Till, B. J., Reynolds, S. H., Enns, L. C., Burtner, C., Johnson, J. E., Odden, A. R., Comai, L. and Henikoff, S. (2003). Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in Arabidopsis. Genetics *164*, 731–740.

[Grün et al., 2006] Grün, S., Lindermayr, C., Sell, S. and Durner, J. (2006). Nitric oxide and gene regulation in plants. J Exp Bot *57*, 507–516.

[Grune et al., 1998] Grune, T., Blasig, I. E., Sitte, N., Roloff, B., Haseloff, R. and Davies, K. J. (1998). Peroxynitrite increases the degradation of aconitase and other cellular proteins by proteasome. J Biol Chem *273*, 10857–10862.

REFERENCES

[Gudesblat et al., 2007] Gudesblat, G. E., Iusem, N. D. and Morris, P. C. (2007). Guard cell-specific inhibition of Arabidopsis MPK3 expression causes abnormal stomatal responses to abscisic acid and hydrogen peroxide. New Phytol *173*, 713–721.

[Guo et al., 2003] Guo, F.-Q., Okamoto, M. and Crawford, N. M. (2003). Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science *302*, 100–103.

[Halliwell, 2006] Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol *141*, 312–322.

[Han et al., 2013] Han, Y., Chaouch, S., Mhamdi, A., Queval, G., Zechmann, B. and Noctor, G. (2013). Functional analysis of Arabidopsis mutants points to novel roles for glutathione in coupling H2O2 to activation of salicylic acid accumulation and signaling. Antioxidants & redox signaling *18*, 2106–2121.

[Hancock et al., 2005] Hancock, J. T., Henson, D., Nyirenda, M., Desikan, R., Harrison, J., Lewis, M., Hughes, J. and Neill, S. J. (2005). Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an inhibitory target of hydrogen peroxide in Arabidopsis. Plant Physiol Biochem *43*, 828–835.

[Hann and Rathjen, 2007] Hann, D. R. and Rathjen, J. P. (2007). Early events in the pathogenicity of Pseudomonas syringae on Nicotiana benthamiana. Plant J *49*, 607–618.

[Hara et al., 2007] Hara, I., Ichise, N., Kojima, K., Kondo, H., Ohgiya, S., Matsuyama, H. and Yumoto, I. (2007). Relationship between the size of the bottleneck 15 A from iron in the main channel and the reactivity of catalase corresponding to the molecular size of substrates. Biochemistry *46*, 11–22.

[Hara et al., 2005] Hara, M. R., Agrawal, N., Kim, S. F., Cascio, M. B., Fujimuro, M., Ozeki, Y., Takahashi, M., Cheah, J. H., Tankou, S. K., Hester, L. D., Ferris, C. D., Hayward, S. D., Snyder, S. H. and Sawa, A. (2005). S-nitrosylated GAPDH initiates apoptotic cell death by nuclear translocation following Siah1 binding. Nat Cell Biol *7*, 665–674.

[Harding et al., 1997] Harding, S. A., Oh, S. H. and Roberts, D. M. (1997). Transgenic tobacco expressing a foreign calmodulin gene shows an enhanced production of active oxygen species. EMBO J *16*, 1137–1144.

[Hauck et al., 2003] Hauck, P., Thilmony, R. and He, S. Y. (2003). A Pseudomonas syringae type III effector suppresses cell wall-based extracellular defense in susceptible Arabidopsis plants. Proc Natl Acad Sci U S A *100*, 8577–8582.

[He et al., 2012] He, J., Duan, Y., Hua, D., Fan, G., Wang, L., Liu, Y., Chen, Z., Han, L., Qu, L.-J. and Gong, Z. (2012). DEXH box RNA helicase-mediated mitochondrial reactive oxygen species production in Arabidopsis mediates crosstalk between abscisic acid and auxin signaling. Plant Cell *24*, 1815–1833.

[He et al., 2004] He, Y., Tang, R.-H., Hao, Y., Stevens, R. D., Cook, C. W., Ahn, S. M., Jing, L., Yang, Z., Chen, L., Guo, F., Fiorani, F., Jackson, R. B., Crawford, N. M. and Pei, Z.-M. (2004). Nitric oxide represses the Arabidopsis floral transition. Science *305*, 1968–1971.

[Heidrich et al., 2011] Heidrich, K., Wirthmueller, L., Tasset, C., Pouzet, C., Deslandes, L. and Parker, J. E. (2011). Arabidopsis EDS1 connects pathogen effector recognition to cell compartment-specific immune responses. Science *334*, 1401–1404.

[Hellens et al., 2000] Hellens, R. P., Edwards, E. A., Leyland, N. R., Bean, S. and Mullineaux, P. M. (2000). pGreen: a versatile and flexible binary Ti vector for Agrobacterium-mediated plant transformation. Plant Mol Biol *42*, 819–832.

[Herbette et al., 2002] Herbette, S., Lenne, C., Leblanc, N., Julien, J.-L., Drevet, J. R. and Roeckel-Drevet, P. (2002). Two GPX-like proteins from Lycopersicon esculentum and Helianthus annuus are antioxidant enzymes with phospholipid hydroperoxide glutathione peroxidase and thioredoxin peroxidase activities. Eur J Biochem *269*, 2414–2420.

[Hess et al., 2005] Hess, D. T., Matsumoto, A., Kim, S.-O., Marshall, H. E. and Stamler, J. S. (2005). Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol *6*, 150–166.

REFERENCES

[Higashi et al., 2008] Higashi, K., Ishiga, Y., Inagaki, Y., Toyoda, K., Shiraishi, T. and Ichinose, Y. (2008). Modulation of defense signal transduction by flagellin-induced WRKY41 transcription factor in Arabidopsis thaliana. Mol Genet Genomics *279*, 303–312.

[Hinsch and Staskawicz, 1996] Hinsch, M. and Staskawicz, B. (1996). Identification of a new Arabidopsis disease resistance locus, RPS4, and cloning of the corresponding avirulence gene, avrRps4, from Pseudomonas syringae pv. pisi. MPMI-Molecular Plant Microbe Interactions *9*, 55–61.

[Hong et al., 2008] Hong, J. K., Yun, B.-W., Kang, J.-G., Raja, M. U., Kwon, E., Sorhagen, K., Chu, C., Wang, Y. and Loake, G. J. (2008). Nitric oxide function and signalling in plant disease resistance. J Exp Bot *59*, 147–154.

[Hotson et al., 2003] Hotson, A., Chosed, R., Shu, H., Orth, K. and Mudgett, M. B. (2003). Xanthomonas type III effector XopD targets SUMO-conjugated proteins in planta. Mol Microbiol *50*, 377–389.

[Hou et al., 2010] Hou, X., Lee, L. Y. C., Xia, K., Yan, Y. and Yu, H. (2010). DELLAs modulate jasmonate signaling via competitive binding to JAZs. Dev Cell *19*, 884–894.

[Howe, 2010] Howe, G. A. (2010). Ubiquitin ligase-coupled receptors extend their reach to jasmonate. Plant Physiol *154*, 471–474.

[Howe and Jander, 2008] Howe, G. A. and Jander, G. (2008). Plant immunity to insect herbivores. Annu Rev Plant Biol *59*, 41–66.

[Hu et al., 2010] Hu, Y.-Q., Liu, S., Yuan, H.-M., Li, J., Yan, D.-W., Zhang, J.-F. and Lu, Y.-T. (2010). Functional comparison of catalase genes in the elimination of photorespiratory H2O2 using promoter- and 3'-untranslated region exchange experiments in the Arabidopsis cat2 photorespiratory mutant. Plant Cell Environ *33*, 1656–1670.

[Huang et al., 2005] Huang, C., He, W., Guo, J., Chang, X., Su, P. and Zhang, L. (2005). Increased sensitivity to salt stress in an ascorbate-deficient Arabidopsis mutant. J Exp Bot *56*, 3041–3049.

[Huang et al., 2004] Huang, X., Stettmaier, K., Michel, C., Hutzler, P., Mueller, M. J. and Durner, J. (2004). Nitric oxide is induced by wounding and influences jasmonic acid signaling in Arabidopsis thaliana. Planta *218*, 938–946.

[Hubert et al., 2003] Hubert, D. A., Tornero, P., Belkhadir, Y., Krishna, P., Takahashi, A., Shirasu, K. and Dangl, J. L. (2003). Cytosolic HSP90 associates with and modulates the Arabidopsis RPM1 disease resistance protein. EMBO J *22*, 5679–5689.

[Iglesias et al., 2010] Iglesias, M. J., Terrile, M. C., Bartoli, C. G., D'Ippólito, S. and Casalongué, C. A. (2010). Auxin signaling participates in the adaptative response against oxidative stress and salinity by interacting with redox metabolism in Arabidopsis. Plant Mol Biol *74*, 215–222.

[Jabs et al., 1996] Jabs, T., Dietrich, R. A. and Dangl, J. L. (1996). Initiation of runaway cell death in an Arabidopsis mutant by extracellular superoxide. Science *273*, 1853–1856.

[Jabs et al., 1997] Jabs, T., Tschope, M., Colling, C., Hahlbrock, K. and Scheel, D. (1997). Elicitor-stimulated ion fluxes and O2- from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. Proc Natl Acad Sci U S A *94*, 4800–4805.

[Jaillais and Chory, 2010] Jaillais, Y. and Chory, J. (2010). Unraveling the paradoxes of plant hormone signaling integration. Nat Struct Mol Biol *17*, 642–645.

[Jakoby et al., 2002] Jakoby, M., Weisshaar, B., Dröge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T., Parcy, F. and , b. I. P. R. G. (2002). bZIP transcription factors in Arabidopsis. Trends Plant Sci *7*, 106–111.

[Jander et al., 2003] Jander, G., Baerson, S. R., Hudak, J. A., Gonzalez, K. A., Gruys, K. J. and Last, R. L. (2003). Ethylmethanesulfonate saturation mutagenesis in Arabidopsis to determine frequency of herbicide resistance. Plant Physiol *131*, 139–146.

REFERENCES

[Jander et al., 2002] Jander, G., Norris, S. R., Rounsley, S. D., Bush, D. F., Levin, I. M. and Last, R. L. (2002). Arabidopsis map-based cloning in the post-genome era. Plant Physiol *129*, 440–450.

[Jelenska et al., 2010] Jelenska, J., van Hal, J. A. and Greenberg, J. T. (2010). Pseudomonas syringae hijacks plant stress chaperone machinery for virulence. Proc Natl Acad Sci U S A *107*, 13177–13182.

[Jia et al., 2000] Jia, Y., McAdams, S. A., Bryan, G. T., Hershey, H. P. and Valent, B. (2000). Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. EMBO J *19*, 4004–4014.

[Jiang et al., 2010] Jiang, C.-J., Shimono, M., Sugano, S., Kojima, M., Yazawa, K., Yoshida, R., Inoue, H., Hayashi, N., Sakakibara, H. and Takatsuji, H. (2010). Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-Magnaporthe grisea interaction. Mol Plant Microbe Interact *23*, 791–798.

[Jones and Dangl, 2006] Jones, J. D. G. and Dangl, J. L. (2006). The plant immune system. Nature *444*, 323–329.

[Joo et al., 2001] Joo, J. H., Bae, Y. S. and Lee, J. S. (2001). Role of auxin-induced reactive oxygen species in root gravitropism. Plant Physiol *126*, 1055–1060.

[Joshi and Loria, 2007] Joshi, M. V. and Loria, R. (2007). Streptomyces turgidiscabies possesses a functional cytokinin biosynthetic pathway and produces leafy galls. Mol Plant Microbe Interact *20*, 751–758.

[Journot-Catalino et al., 2006] Journot-Catalino, N., Somssich, I. E., Roby, D. and Kroj, T. (2006). The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in Arabidopsis thaliana. Plant Cell *18*, 3289–3302.

[Jung et al., 2002] Jung, B. G., Lee, K. O., Lee, S. S., Chi, Y. H., Jang, H. H., Kang, S. S., Lee, K., Lim, D., Yoon, S. C., Yun, D.-J., Inoue, Y., Cho, M. J. and Lee, S. Y. (2002). A Chinese cabbage cDNA with high sequence identity to phospholipid hydroperoxide glutathione peroxidases encodes a novel isoform of thioredoxin-dependent peroxidase. J Biol Chem *277*, 12572–12578.

[Kachroo et al., 2003] Kachroo, A., Lapchyk, L., Fukushige, H., Hildebrand, D., Klessig, D. and Kachroo, P. (2003). Plastidial fatty acid signaling modulates salicylic acid- and jasmonic acid-mediated defense pathways in the Arabidopsis ssi2 mutant. Plant Cell *15*, 2952–2965.

[Kachroo et al., 2001] Kachroo, P., Shanklin, J., Shah, J., Whittle, E. J. and Klessig, D. F. (2001). A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc Natl Acad Sci U S A *98*, 9448–9453.

[Kajava, 1998] Kajava, A. V. (1998). Structural diversity of leucine-rich repeat proteins. J Mol Biol *277*, 519–527.

[Kaminaka et al., 2006] Kaminaka, H., Näke, C., Epple, P., Dittgen, J., Schütze, K., Chaban, C., Holt, 3rd, B. F., Merkle, T., Schäfer, E., Harter, K. and Dangl, J. L. (2006). bZIP10-LSD1 antagonism modulates basal defense and cell death in Arabidopsis following infection. EMBO J *25*, 4400–4411.

[Kamoun et al., 1998] Kamoun, S., van der Lee, T., van den Berg-Velthuis, G., de Groot, K. E. and Govers, F. (1998). Loss of Production of the Elicitor Protein INF1 in the Clonal Lineage US-1 of Phytophthora infestans. Phytopathology *88*, 1315–1323.

[Katagiri, 2004] Katagiri, F. (2004). A global view of defense gene expression regulation–a highly interconnected signaling network. Curr Opin Plant Biol *7*, 506–511.

[Katagiri and Tsuda, 2010] Katagiri, F. and Tsuda, K. (2010). Understanding the plant immune system. Mol Plant Microbe Interact *23*, 1531–1536.

[Katsir et al., 2008] Katsir, L., Schilmiller, A. L., Staswick, P. E., He, S. Y. and Howe, G. A. (2008). COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. Proc Natl Acad Sci U S A *105*, 7100–7105.

REFERENCES

[Kazan, 2006] Kazan, K. (2006). Negative regulation of defence and stress genes by EAR-motif-containing repressors. Trends Plant Sci *11*, 109–112.

[Kazan and Manners, 2009] Kazan, K. and Manners, J. M. (2009). Linking development to defense: auxin in plant-pathogen interactions. Trends Plant Sci *14*, 373–382.

[Kelley and Sternberg, 2009] Kelley, L. A. and Sternberg, M. J. E. (2009). Protein structure prediction on the Web: a case study using the Phyre server. Nat Protoc *4*, 363–371.

[Kesarwani et al., 2007] Kesarwani, M., Yoo, J. and Dong, X. (2007). Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. Plant Physiol *144*, 336–346.

[Kim et al., 2005] Kim, H.-S., Desveaux, D., Singer, A. U., Patel, P., Sondek, J. and Dangl, J. L. (2005). The Pseudomonas syringae effector AvrRpt2 cleaves its C-terminally acylated target, RIN4, from Arabidopsis membranes to block RPM1 activation. Proc Natl Acad Sci U S A *102*, 6496–6501.

[Kim et al., 2008] Kim, K.-C., Lai, Z., Fan, B. and Chen, Z. (2008). Arabidopsis WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. Plant Cell *20*, 2357–2371.

[Kim et al., 2009] Kim, S. H., Kwon, S. I., Saha, D., Anyanwu, N. C. and Gassmann, W. (2009). Resistance to the Pseudomonas syringae effector HopA1 is governed by the TIR-NBS-LRR protein RPS6 and is enhanced by mutations in SRFR1. Plant Physiol *150*, 1723–1732.

[Kim et al., 2006] Kim, Y., Schumaker, K. S. and Zhu, J.-K. (2006). EMS mutagenesis of Arabidopsis. In Arabidopsis Protocols pp. 101–103. Springer.

[Kinkema et al., 2000] Kinkema, M., Fan, W. and Dong, X. (2000). Nuclear localization of NPR1 is required for activation of PR gene expression. Plant Cell *12*, 2339–2350.

[Klessig et al., 2000] Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne, D., Kumar, D., Zhou, J. M., Shah, J., Zhang, S., Kachroo, P., Trifa, Y., Pontier, D., Lam, E. and Silva, H. (2000). Nitric oxide and salicylic acid signaling in plant defense. Proc Natl Acad Sci U S A *97*, 8849–8855.

[Kliebenstein et al., 1999] Kliebenstein, D. J., Dietrich, R. A., Martin, A. C., Last, R. L. and Dangl, J. L. (1999). LSD1 regulates salicylic acid induction of copper zinc superoxide dismutase in Arabidopsis thaliana. Mol Plant Microbe Interact *12*, 1022–1026.

[Knoth et al., 2007] Knoth, C., Ringler, J., Dangl, J. L. and Eulgem, T. (2007). Arabidopsis WRKY70 is required for full RPP4-mediated disease resistance and basal defense against Hyaloperonospora parasitica. Mol Plant Microbe Interact *20*, 120–128.

[Kolisek et al., 2005] Kolisek, M., Beck, A., Fleig, A. and Penner, R. (2005). Cyclic ADP-ribose and hydrogen peroxide synergize with ADP-ribose in the activation of TRPM2 channels. Mol Cell *18*, 61–69.

[Koo et al., 2009] Koo, A. J. K., Gao, X., Jones, A. D. and Howe, G. A. (2009). A rapid wound signal activates the systemic synthesis of bioactive jasmonates in Arabidopsis. Plant J *59*, 974–986.

[Koornneef et al., 2008] Koornneef, A., Leon-Reyes, A., Ritsema, T., Verhage, A., Den Otter, F. C., Van Loon, L. C. and Pieterse, C. M. J. (2008). Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiol *147*, 1358–1368.

[Koornneef and Pieterse, 2008] Koornneef, A. and Pieterse, C. M. J. (2008). Cross talk in defense signaling. Plant Physiol *146*, 839–844.

[Kovtun et al., 2000] Kovtun, Y., Chiu, W. L., Tena, G. and Sheen, J. (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc Natl Acad Sci U S A *97*, 2940–2945.

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

REFERENCES

[Kunze et al., 2004] Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T. and Felix, G. (2004). The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. Plant Cell *16*, 3496–3507.

[Kwon et al., 2009] Kwon, S. I., Kim, S. H., Bhattacharjee, S., Noh, J.-J. and Gassmann, W. (2009). SRFR1, a suppressor of effector-triggered immunity, encodes a conserved tetratricopeptide repeat protein with similarity to transcriptional repressors. Plant J *57*, 109–119.

[Lackman et al., 2011] Lackman, P., González-Guzmán, M., Tilleman, S., Carqueijeiro, I., Pérez, A. C., Moses, T., Seo, M., Kanno, Y., Häkkinen, S. T., Van Montagu, M. C. E., Thevelein, J. M., Maaheimo, H., Oksman-Caldentey, K.-M., Rodriguez, P. L., Rischer, H. and Goossens, A. (2011). Jasmonate signaling involves the abscisic acid receptor PYL4 to regulate metabolic reprogramming in Arabidopsis and tobacco. Proc Natl Acad Sci U S A *108*, 5891–5896.

[Laibach, 1943] Laibach, F. (1943). Arabidopsis thaliana (L.) Heynh. als Objekt fuer genetische und entwicklungsphysiologische Untersuchungen. Bot. Archiv *44*, 439–455.

[Laird et al., 2004] Laird, J., Armengaud, P., Giuntini, P., Laval, V. and Milner, J. J. (2004). Inappropriate annotation of a key defence marker in Arabidopsis: will the real PR-1 please stand up? Planta *219*, 1089–1092.

[Laloi et al., 2004] Laloi, C., Mestres-Ortega, D., Marco, Y., Meyer, Y. and Reichheld, J.-P. (2004). The Arabidopsis cytosolic thioredoxin h5 gene induction by oxidative stress and its W-box-mediated response to pathogen elicitor. Plant Physiol *134*, 1006–1016.

[Lam et al., 2001] Lam, E., Kato, N. and Lawton, M. (2001). Programmed cell death, mitochondria and the plant hypersensitive response. Nature *411*, 848–853.

[Lamattina et al., 2003] Lamattina, L., García-Mata, C., Graziano, M. and Pagnussat, G. (2003). Nitric oxide: the versatility of an extensive signal molecule. Annu Rev Plant Biol *54*, 109–136.

[Lamb and Dixon, 1997] Lamb, C. and Dixon, R. A. (1997). The oxidative burst in plant disease resistance. Annual review of plant biology *48*, 251–275.

[Larkindale et al., 2005] Larkindale, J., Hall, J. D., Knight, M. R. and Vierling, E. (2005). Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol *138*, 882–897.

[Lebel et al., 1998] Lebel, E., Heifetz, P., Thorne, L., Uknes, S., Ryals, J. and Ward, E. (1998). Functional analysis of regulatory sequences controlling PR-1 gene expression in Arabidopsis. Plant J *16*, 223–233.

[Lee et al., 2008] Lee, U., Wie, C., Fernandez, B. O., Feelisch, M. and Vierling, E. (2008). Modulation of nitrosative stress by S-nitrosoglutathione reductase is critical for thermotolerance and plant growth in Arabidopsis. Plant Cell *20*, 786–802.

[Leitner et al., 2009] Leitner, M., Vandelle, E., Gaupels, F., Bellin, D. and Delledonne, M. (2009). NO signals in the haze: nitric oxide signalling in plant defence. Curr Opin Plant Biol *12*, 451–458.

[Lemaitre et al., 1997] Lemaitre, B., Reichhart, J. M. and Hoffmann, J. A. (1997). Drosophila host defense: differential induction of antimicrobial peptide genes after infection by various classes of microorganisms. Proc Natl Acad Sci U S A *94*, 14614–14619.

[Leng et al., 1999] Leng, Q., Mercier, R. W., Yao, W. and Berkowitz, G. A. (1999). Cloning and first functional characterization of a plant cyclic nucleotide-gated cation channel. Plant Physiology *121*, 753–761.

[Leon-Reyes et al., 2010] Leon-Reyes, A., Du, Y., Koornneef, A., Proietti, S., Körbes, A. P., Memelink, J., Pieterse, C. M. J. and Ritsema, T. (2010). Ethylene signaling renders the jasmonate response of Arabidopsis insensitive to future suppression by salicylic Acid. Mol Plant Microbe Interact *23*, 187–197.

REFERENCES

[Leon-Reyes et al., 2009] Leon-Reyes, A., Spoel, S. H., De Lange, E. S., Abe, H., Kobayashi, M., Tsuda, S., Millenaar, F. F., Welschen, R. A. M., Ritsema, T. and Pieterse, C. M. J. (2009). Ethylene modulates the role of NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 in cross talk between salicylate and jasmonate signaling. Plant Physiol *149*, 1797–1809.

[Leyser, 2003] Leyser, O. (2003). Regulation of shoot branching by auxin. Trends Plant Sci *8*, 541–545.

[Li et al., 2006] Li, J., Brader, G., Kariola, T. and Palva, E. T. (2006). WRKY70 modulates the selection of signaling pathways in plant defense. Plant J *46*, 477–491.

[Li et al., 2004] Li, J., Brader, G. and Palva, E. T. (2004). The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell *16*, 319–331.

[Li et al., 1999] Li, X., Zhang, Y., Clarke, J. D., Li, Y. and Dong, X. (1999). Identification and Cloning of a Negative Regulator of Systemic Acquired Resistance, SNI1, through a Screen for Suppressors of npr1-1. Cell *98*, 329–339.

[Ligterink et al., 1997] Ligterink, W., Kroj, T., zur Nieden, U., Hirt, H. and Scheel, D. (1997). Receptor-mediated activation of a MAP kinase in pathogen defense of plants. Science *276*, 2054–2057.

[Lillig and Holmgren, 2007] Lillig, C. H. and Holmgren, A. (2007). Thioredoxin and related molecules–from biology to health and disease. Antioxid Redox Signal *9*, 25–47.

[Lin et al., 2012] Lin, A., Wang, Y., Tang, J., Xue, P., Li, C., Liu, L., Hu, B., Yang, F., Loake, G. J. and Chu, C. (2012). Nitric oxide and protein S-nitrosylation are integral to hydrogen peroxide-induced leaf cell death in rice. Plant Physiol *158*, 451–464.

[Lindermayr et al., 2006] Lindermayr, C., Saalbach, G., Bahnweg, G. and Durner, J. (2006). Differential inhibition of Arabidopsis methionine adenosyltransferases by protein S-nitrosylation. J Biol Chem *281*, 4285–4291.

[Lindermayr et al., 2005] Lindermayr, C., Saalbach, G. and Durner, J. (2005). Proteomic identification of S-nitrosylated proteins in Arabidopsis. Plant Physiol *137*, 921–930.

[Lindermayr et al., 2010] Lindermayr, C., Sell, S., Müller, B., Leister, D. and Durner, J. (2010). Redox regulation of the NPR1-TGA1 system of Arabidopsis thaliana by nitric oxide. Plant Cell *22*, 2894–2907.

[Liu et al., 2011] Liu, J., Elmore, J. M., Lin, Z.-J. D. and Coaker, G. (2011). A receptor-like cytoplasmic kinase phosphorylates the host target RIN4, leading to the activation of a plant innate immune receptor. Cell Host Microbe *9*, 137–146.

[Liu et al., 2001] 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.

[Llorente et al., 2005] Llorente, F., Alonso-Blanco, C., Sánchez-Rodriguez, C., Jorda, L. and Molina, A. (2005). ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus Plectosphaerella cucumerina. Plant J *43*, 165–180.

[Loake and Grant, 2007] Loake, G. and Grant, M. (2007). Salicylic acid in plant defence–the players and protagonists. Curr Opin Plant Biol *10*, 466–472.

[Lorenzo et al., 2004] Lorenzo, O., Chico, J. M., Sánchez-Serrano, J. J. and Solano, R. (2004). JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. Plant Cell *16*, 1938–1950.

[Lorenzo et al., 2003] Lorenzo, O., Piqueras, R., Sánchez-Serrano, J. J. and Solano, R. (2003). ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell *15*, 165–178.

REFERENCES

[Lukowitz et al., 2000] Lukowitz, W., Gillmor, C. S. and Scheible, W. R. (2000). Positional cloning in Arabidopsis. Why it feels good to have a genome initiative working for you. Plant Physiol *123*, 795–805.

[Mackey et al., 2002] Mackey, D., Holt, 3rd, B. F., Wiig, A. and Dangl, J. L. (2002). RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell *108*, 743–754.

[Maldonado-Alconada et al., 2011] Maldonado-Alconada, A. M., Echevarría-Zomeño, S., Lindermayr, C., Redondo-López, I., Durner, J. and Jorrín-Novo, J. V. (2011). Proteomic analysis of Arabidopsis protein S-nitrosylation in response to inoculation with Pseudomonas syringae. Acta Physiologiae Plantarum *33*, 1493–1514.

[Malik et al., 2011] Malik, S. I., Hussain, A., Yun, B.-W., Spoel, S. H. and Loake, G. J. (2011). GSNOR-mediated de-nitrosylation in the plant defence response. Plant Sci *181*, 540–544.

[Mao et al., 2007] Mao, P., Duan, M., Wei, C. and Li, Y. (2007). WRKY62 transcription factor acts downstream of cytosolic NPR1 and negatively regulates jasmonate-responsive gene expression. Plant Cell Physiol *48*, 833–842.

[Marchand et al., 2004] Marchand, C., Le Maréchal, P., Meyer, Y., Miginiac-Maslow, M., Issakidis-Bourguet, E. and Decottignies, P. (2004). New targets of Arabidopsis thioredoxins revealed by proteomic analysis. Proteomics *4*, 2696–2706.

[Marois et al., 2002] Marois, E., Van den Ackerveken, G. and Bonas, U. (2002). The xanthomonas type III effector protein AvrBs3 modulates plant gene expression and induces cell hypertrophy in the susceptible host. Mol Plant Microbe Interact *15*, 637–646.

[Marumo et al., 1982] Marumo, S., Katayama, M., Komori, E., Ozaki, Y., Natsume, M. and Kondo, S. (1982). Microbial Production of Abscisic Acid by Botrytis cinerea. Agricultural and Biological Chemistry *46*, 1967–1968.

[Mateo et al., 2004] Mateo, A., Mühlenbock, P., Rustérucci, C., Chang, C. C.-C., Miszalski, Z., Karpinska, B., Parker, J. E., Mullineaux, P. M. and Karpinski, S. (2004). LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy. Plant Physiol *136*, 2818–2830.

[McDermott et al., 2000] McDermott, P. F., Ciacci-Woolwine, F., Snipes, J. A. and Mizel, S. B. (2000). High-affinity interaction between gram-negative flagellin and a cell surface polypeptide results in human monocyte activation. Infect Immun *68*, 5525–5529.

[McDowell et al., 2000] McDowell, J. M., Cuzick, A., Can, C., Beynon, J., Dangl, J. L. and Holub, E. B. (2000). Downy mildew (Peronospora parasitica) resistance genes in Arabidopsis vary in functional requirements for NDR1, EDS1, NPR1 and salicylic acid accumulation. Plant J *22*, 523–529.

[McGrath et al., 2005] McGrath, K. C., Dombrecht, B., Manners, J. M., Schenk, P. M., Edgar, C. I., Maclean, D. J., Scheible, W.-R., Udvardi, M. K. and Kazan, K. (2005). Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression. Plant Physiol *139*, 949–959.

[Meinke et al., 1998] Meinke, D. W., Cherry, J. M., Dean, C., Rounsley, S. D. and Koornneef, M. (1998). Arabidopsis thaliana: a model plant for genome analysis. Science *282*, 662, 679–662, 682.

[Melotto et al., 2008] Melotto, M., Underwood, W. and He, S. Y. (2008). Role of stomata in plant innate immunity and foliar bacterial diseases. Annu Rev Phytopathol *46*, 101–122.

[Melotto et al., 2006] Melotto, M., Underwood, W., Koczan, J., Nomura, K. and He, S. Y. (2006). Plant stomata function in innate immunity against bacterial invasion. Cell *126*, 969–980.

[Memelink, 2009] Memelink, J. (2009). Regulation of gene expression by jasmonate hormones. Phytochemistry *70*, 1560–1570.

REFERENCES

[Mersmann et al., 2008] Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J. W. and Robatzek, S. (2008). Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. Current Biology *18*, 1824–1832.

[Meyer et al., 2005] Meyer, Y., Reichheld, J. P. and Vignols, F. (2005). Thioredoxins in Arabidopsis and other plants. Photosynth Res *86*, 419–433.

[Mhamdi et al., 2012] Mhamdi, A., Noctor, G. and Baker, A. (2012). Plant catalases: peroxisomal redox guardians. Arch Biochem Biophys *525*, 181–194.

[Mhamdi et al., 2010] Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Van Breusegem, F. and Noctor, G. (2010). Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. J Exp Bot *61*, 4197–4220.

[Mühlenbock et al., 2008] Mühlenbock, P., Szechynska-Hebda, M., Plaszczyca, M., Baudo, M., Mateo, A., Mullineaux, P. M., Parker, J. E., Karpinska, B. and Karpinski, S. (2008). Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in Arabidopsis. Plant Cell *20*, 2339–2356.

[Michelmore et al., 1991] Michelmore, R. W., Paran, I. and Kesseli, R. V. (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci U S A *88*, 9828–9832.

[Millet et al., 2010] Millet, Y. A., Danna, C. H., Clay, N. K., Songnuan, W., Simon, M. D., Werck-Reichhart, D. and Ausubel, F. M. (2010). Innate immune responses activated in Arabidopsis roots by microbe-associated molecular patterns. Plant Cell *22*, 973–990.

[Mishina and Zeier, 2006] Mishina, T. E. and Zeier, J. (2006). The Arabidopsis flavin-dependent monooxygenase FMO1 is an essential component of biologically induced systemic acquired resistance. Plant Physiol *141*, 1666–1675.

[Mittler, 2002] Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci *7*, 405–410.

[Mittler et al., 1998] Mittler, R., Feng, X. and Cohen, M. (1998). Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. Plant Cell *10*, 461–473.

[Mittler et al., 1999] Mittler, R., Herr, E. H., Orvar, B. L., van Camp, W., Willekens, H., Inzé, D. and Ellis, B. E. (1999). Transgenic tobacco plants with reduced capability to detoxify reactive oxygen intermediates are hyperresponsive to pathogen infection. Proc Natl Acad Sci U S A *96*, 14165–14170.

[Mittler et al., 2004] Mittler, R., Vanderauwera, S., Gollery, M. and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. Trends Plant Sci *9*, 490–498.

[Modolo et al., 2005] Modolo, L. V., Augusto, O., Almeida, I. M. G., Magalhaes, J. R. and Salgado, I. (2005). Nitrite as the major source of nitric oxide production by Arabidopsis thaliana in response to Pseudomonas syringae. FEBS Lett *579*, 3814–3820.

[Monaghan et al., 2010] Monaghan, J., Germain, H., Weihmann, T. and Li, X. (2010). Dissecting plant defence signal transduction: modifiers of snc1 in Arabidopsis. Canadian Journal of Plant Pathology *32*, 35–42.

[Moon et al., 2003] Moon, H., Lee, B., Choi, G., Shin, D., Prasad, D. T., Lee, O., Kwak, S.-S., Kim, D. H., Nam, J., Bahk, J., Hong, J. C., Lee, S. Y., Cho, M. J., Lim, C. O. and Yun, D.-J. (2003). NDP kinase 2 interacts with two oxidative stress-activated MAPKs to regulate cellular redox state and enhances multiple stress tolerance in transgenic plants. Proc Natl Acad Sci U S A *100*, 358–363.

[Moon et al., 2004] Moon, J., Parry, G. and Estelle, M. (2004). The ubiquitin-proteasome pathway and plant development. Plant Cell *16*, 3181–3195.

[Moore et al., 2011] Moore, J. W., Loake, G. J. and Spoel, S. H. (2011). Transcription dynamics in plant immunity. Plant Cell *23*, 2809–2820.

REFERENCES

[Moreau et al., 2010] Moreau, M., Lindermayr, C., Durner, J. and Klessig, D. F. (2010). NO synthesis and signaling in plants–where do we stand? Physiol Plant *138*, 372–383.

[Mou et al., 2003] Mou, Z., Fan, W. and Dong, X. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell *113*, 935–944.

[Mudgett, 2005] Mudgett, M. B. (2005). New insights to the function of phytopathogenic bacterial type III effectors in plants. Annu Rev Plant Biol *56*, 509–531.

[Mueller-Moule et al., 2003] Mueller-Moule, P., Havaux, M. and Niyogi, K. K. (2003). Zeaxanthin deficiency enhances the high light sensitivity of an ascorbate-deficient mutant of Arabidopsis. Plant Physiol *133*, 748–760.

[Mundy et al., 2006] Mundy, J., Nielsen, H. B. and Brodersen, P. (2006). Crosstalk. Trends Plant Sci *11*, 63–64.

[Mur et al., 2006a] Mur, L. A. J., Carver, T. L. W. and Prats, E. (2006a). NO way to live; the various roles of nitric oxide in plant-pathogen interactions. J Exp Bot *57*, 489–505.

[Mur et al., 2006b] Mur, L. A. J., Kenton, P., Atzorn, R., Miersch, O. and Wasternack, C. (2006b). The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. Plant Physiol *140*, 249–262.

[Mur et al., 2008a] Mur, L. A. J., Kenton, P., Lloyd, A. J., Ougham, H. and Prats, E. (2008a). The hypersensitive response; the centenary is upon us but how much do we know? J Exp Bot *59*, 501–520.

[Mur et al., 2008b] Mur, L. A. J., Laarhoven, L. J. J., Harren, F. J. M., Hall, M. A. and Smith, A. R. (2008b). Nitric oxide interacts with salicylate to regulate biphasic ethylene production during the hypersensitive response. Plant Physiol *148*, 1537–1546.

[Nakashita et al., 2003] Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujioka, S., Arai, Y., Sekimata, K., Takatsuto, S., Yamaguchi, I. and Yoshida, S. (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. The Plant Journal *33*, 887–898.

[Nathan and Xie, 1994] Nathan, C. and Xie, Q. W. (1994). Nitric oxide synthases: roles, tolls, and controls. Cell *78*, 915–918.

[Navarro et al., 2008] Navarro, L., Bari, R., Achard, P., Lisón, P., Nemri, A., Harberd, N. P. and Jones, J. D. G. (2008). DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signaling. Curr Biol *18*, 650–655.

[Navarro et al., 2006] Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. and Jones, J. D. G. (2006). A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science *312*, 436–439.

[Nawrath and Metraux, 1999] Nawrath, C. and Metraux, J. P. (1999). Salicylic acid induction-deficient mutants of Arabidopsis express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. Plant Cell *11*, 1393–1404.

[Ndamukong et al., 2007] Ndamukong, I., Abdallat, A. A., Thurow, C., Fode, B., Zander, M., Weigel, R. and Gatz, C. (2007). SA-inducible Arabidopsis glutaredoxin interacts with TGA factors and suppresses JA-responsive PDF1.2 transcription. Plant J *50*, 128–139.

[Neill et al., 2002] Neill, S., Desikan, R. and Hancock, J. (2002). Hydrogen peroxide signalling. Curr Opin Plant Biol *5*, 388–395.

[Nühse et al., 2007] Nühse, T. S., Bottrill, A. R., Jones, A. M. E. and Peck, S. C. (2007). Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J *51*, 931–940.

[Niu et al., 2011] Niu, Y., Figueroa, P. and Browse, J. (2011). Characterization of JAZ-interacting bHLH transcription factors that regulate jasmonate responses in Arabidopsis. J Exp Bot *62*, 2143–2154.

REFERENCES

[Noctor, 2006] Noctor, G. (2006). Metabolic signalling in defence and stress: the central roles of soluble redox couples. Plant Cell Environ *29*, 409–425.

[Nomura et al., 2005] Nomura, K., Melotto, M. and He, S.-Y. (2005). Suppression of host defense in compatible plant-Pseudomonas syringae interactions. Curr Opin Plant Biol *8*, 361–368.

[Nürnberger et al., 2004] Nürnberger, T., Brunner, F., Kemmerling, B. and Piater, L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev *198*, 249–266.

[O'Brien et al., 2012a] O'Brien, J. A., Daudi, A., Butt, V. S. and Bolwell, G. P. (2012a). Reactive oxygen species and their role in plant defence and cell wall metabolism. Planta *236*, 765–779.

[O'Brien et al., 2012b] O'Brien, J. A., Daudi, A., Finch, P., Butt, V. S., Whitelegge, J. P., Souda, P., Ausubel, F. M. and Bolwell, G. P. (2012b). A peroxidase-dependent apoplastic oxidative burst in cultured Arabidopsis cells functions in MAMP-elicited defense. Plant Physiol *158*, 2013–2027.

[O'Donnell et al., 2003a] O'Donnell, P. J., Schmelz, E. A., Moussatche, P., Lund, S. T., Jones, J. B. and Klee, H. J. (2003a). Susceptible to intolerance–a range of hormonal actions in a susceptible Arabidopsis pathogen response. The Plant Journal *33*, 245–257.

[O'Donnell et al., 2003b] O'Donnell, P. J., Schmelz, E. A., Moussatche, P., Lund, S. T., Jones, J. B. and Klee, H. J. (2003b). Susceptible to intolerance–a range of hormonal actions in a susceptible Arabidopsis pathogen response. Plant J *33*, 245–257.

[Ogawa et al., 2005] Ogawa, T., Ueda, Y., Yoshimura, K. and Shigeoka, S. (2005). Comprehensive analysis of cytosolic Nudix hydrolases in Arabidopsis thaliana. J Biol Chem *280*, 25277–25283.

[Okada et al., 1991] Okada, K., Ueda, J., Komaki, M. K., Bell, C. J. and Shimura, Y. (1991). Requirement of the Auxin Polar Transport System in Early Stages of Arabidopsis Floral Bud Formation. Plant Cell *3*, 677–684.

[Okrent et al., 2009] Okrent, R. A., Brooks, M. D. and Wildermuth, M. C. (2009). Arabidopsis GH3.12 (PBS3) conjugates amino acids to 4-substituted benzoates and is inhibited by salicylate. J Biol Chem *284*, 9742–9754.

[Oliveira et al., 2010] Oliveira, H. C., Saviani, E. E., Oliveira, J. F. and Salgado, I. (2010). Nitrate reductase-dependent nitric oxide synthesis in the defense response of Arabidopsis thaliana against Pseudomonas syringae. Tropical Plant Pathology *35*, 104–107.

[Olszak et al., 2006] Olszak, B., Malinovsky, F. G., Brodersen, P., Grell, M., Giese, H., Petersen, M. and Mundy, J. (2006). A putative flavin-containing mono-oxygenase as a marker for certain defense and cell death pathways. Plant Science *170*, 614–623.

[Orendi et al., 2001] Orendi, G., Zimmermann, P., Baar, C. and Zentgraf, U. (2001). Loss of stress-induced expression of catalase3 during leaf senescence in Arabidopsis thaliana is restricted to oxidative stress. Plant Sci *161*, 301–314.

[Overmyer et al., 2003] Overmyer, K., Brosché, M. and Kangasjärvi, J. (2003). Reactive oxygen species and hormonal control of cell death. Trends Plant Sci *8*, 335–342.

[Page and Grossniklaus, 2002] Page, D. R. and Grossniklaus, U. (2002). The art and design of genetic screens: Arabidopsis thaliana. Nat Rev Genet *3*, 124–136.

[Palmer et al., 1993] Palmer, R. M., Hickery, M. S., Charles, I. G., Moncada, S. and Bayliss, M. T. (1993). Induction of nitric oxide synthase in human chondrocytes. Biochem Biophys Res Commun *193*, 398–405.

[Palmieri et al., 2010] Palmieri, M. C., Lindermayr, C., Bauwe, H., Steinhauser, C. and Durner, J. (2010). Regulation of plant glycine decarboxylase by s-nitrosylation and glutathionylation. Plant Physiol *152*, 1514–1528.

REFERENCES

[Pape et al., 2010] Pape, S., Thurow, C. and Gatz, C. (2010). The Arabidopsis PR-1 promoter contains multiple integration sites for the coactivator NPR1 and the repressor SNI1. Plant Physiol *154*, 1805–1818.

[Parisy et al., 2007] Parisy, V., Poinssot, B., Owsianowski, L., Buchala, A., Glazebrook, J. and Mauch, F. (2007). Identification of PAD2 as a gamma-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis. Plant J *49*, 159–172.

[Park et al., 2007] Park, S.-W., Kaimoyo, E., Kumar, D., Mosher, S. and Klessig, D. F. (2007). Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science *318*, 113–116.

[Parker et al., 1996] Parker, J. E., Holub, E. B., Frost, L. N., Falk, A., Gunn, N. D. and Daniels, M. J. (1996). Characterization of eds1, a mutation in Arabidopsis suppressing resistance to Peronospora parasitica specified by several different RPP genes. Plant Cell *8*, 2033–2046.

[Pauwels et al., 2010] Pauwels, L., Barbero, G. F., Geerinck, J., Tilleman, S., Grunewald, W., Pérez, A. C., Chico, J. M., Bossche, R. V., Sewell, J., Gil, E., García-Casado, G., Witters, E., Inzé, D., Long, J. A., De Jaeger, G., Solano, R. and Goossens, A. (2010). NINJA connects the co-repressor TOPLESS to jasmonate signalling. Nature *464*, 788–791.

[Pauwels and Goossens, 2011] Pauwels, L. and Goossens, A. (2011). The JAZ proteins: a crucial interface in the jasmonate signaling cascade. Plant Cell *23*, 3089–3100.

[Pavet et al., 2005] Pavet, V., Olmos, E., Kiddle, G., Mowla, S., Kumar, S., Antoniw, J., Alvarez, M. E. and Foyer, C. H. (2005). Ascorbic acid deficiency activates cell death and disease resistance responses in Arabidopsis. Plant Physiol *139*, 1291–1303.

[Peck et al., 2001] Peck, S. C., Nühse, T. S., Hess, D., Iglesias, A., Meins, F. and Boller, T. (2001). Directed proteomics identifies a plant-specific protein rapidly phosphorylated in response to bacterial and fungal elicitors. Plant Cell *13*, 1467–1475.

[Peer and Murphy, 2006] Peer, W. and Murphy, A. S. (2006). Flavonoids as signal molecules: targets of flavonoid action. In The science of flavonoids pp. 239–268. Springer.

[Peer et al., 2013] Peer, W. A., Cheng, Y. and Murphy, A. S. (2013). Evidence of oxidative attenuation of auxin signalling. J Exp Bot *64*, 2629–2639.

[Pei et al., 2000] Pei, Z. M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G. J., Grill, E. and Schroeder, J. I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature *406*, 731–734.

[Peng et al., 1999] Peng, J., Richards, D. E., Moritz, T., Caño-Delgado, A. and Harberd, N. P. (1999). Extragenic suppressors of the Arabidopsis gai mutation alter the dose-response relationship of diverse gibberellin responses. Plant Physiol *119*, 1199–1208.

[Perazzolli et al., 2004] Perazzolli, M., Dominici, P., Romero-Puertas, M. C., Zago, E., Zeier, J., Sonoda, M., Lamb, C. and Delledonne, M. (2004). Arabidopsis nonsymbiotic hemoglobin AHb1 modulates nitric oxide bioactivity. Plant Cell *16*, 2785–2794.

[Perraud et al., 2001] Perraud, A. L., Fleig, A., Dunn, C. A., Bagley, L. A., Launay, P., Schmitz, C., Stokes, A. J., Zhu, Q., Bessman, M. J., Penner, R., Kinet, J. P. and Scharenberg, A. M. (2001). ADP-ribose gating of the calcium-permeable LTRPC2 channel revealed by Nudix motif homology. Nature *411*, 595–599.

[Perraud et al., 2005] Perraud, A.-L., Takanishi, C. L., Shen, B., Kang, S., Smith, M. K., Schmitz, C., Knowles, H. M., Ferraris, D., Li, W., Zhang, J., Stoddard, B. L. and Scharenberg, A. M. (2005). Accumulation of free ADP-ribose from mitochondria mediates oxidative stress-induced gating of TRPM2 cation channels. J Biol Chem *280*, 6138–6148.

[Petersen et al., 2000] Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H. B., Lacy, M., Austin, M. J., Parker, J. E., Sharma, S. B., Klessig, D. F., Martienssen, R., Mattsson, O., Jensen, A. B. and Mundy, J. (2000). Arabidopsis map kinase 4 negatively regulates systemic acquired resistance. Cell *103*, 1111–1120.

REFERENCES

[Pieterse et al., 2009] Pieterse, C. M. J., Leon-Reyes, A., Van der Ent, S. and Van Wees, S. C. M. (2009). Networking by small-molecule hormones in plant immunity. Nat Chem Biol *5*, 308–316.

[Pieterse et al., 2012] Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A. and Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol *28*, 489–521.

[Pieterse and Van Loon, 2004] Pieterse, C. M. J. and Van Loon, L. C. (2004). NPR1: the spider in the web of induced resistance signaling pathways. Curr Opin Plant Biol *7*, 456–464.

[Pineda et al., 2010] Pineda, A., Zheng, S.-J., van Loon, J. J. A., Pieterse, C. M. J. and Dicke, M. (2010). Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends Plant Sci *15*, 507–514.

[Pitzschke et al., 2009] Pitzschke, A., Schikora, A. and Hirt, H. (2009). MAPK cascade signalling networks in plant defence. Curr Opin Plant Biol *12*, 421–426.

[Planchet et al., 2005] Planchet, E., Jagadis Gupta, K., Sonoda, M. and Kaiser, W. M. (2005). Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J *41*, 732–743.

[Pré et al., 2008] Pré, M., Atallah, M., Champion, A., De Vos, M., Pieterse, C. M. J. and Memelink, J. (2008). The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiol *147*, 1347–1357.

[Putnam et al., 2000] Putnam, C. D., Arvai, A. S., Bourne, Y. and Tainer, J. A. (2000). Active and inhibited human catalase structures: ligand and NADPH binding and catalytic mechanism. J Mol Biol *296*, 295–309.

[Qiu et al., 2008] Qiu, J.-L., Fiil, B. K., Petersen, K., Nielsen, H. B., Botanga, C. J., Thorgrimsen, S., Palma, K., Suarez-Rodriguez, M. C., Sandbech-Clausen, S., Lichota, J., Brodersen, P., Grasser, K. D., Mattsson, O., Glazebrook, J., Mundy, J. and Petersen, M. (2008). Arabidopsis MAP kinase 4 regulates gene expression through transcription factor release in the nucleus. EMBO J *27*, 2214–2221.

[Queval et al., 2008] Queval, G., Hager, J., Gakière, B. and Noctor, G. (2008). Why are literature data for H2O2 contents so variable? A discussion of potential difficulties in the quantitative assay of leaf extracts. J Exp Bot *59*, 135–146.

[Queval et al., 2007] Queval, G., Issakidis-Bourguet, E., Hoeberichts, F. A., Vandorpe, M., Gakière, B., Vanacker, H., Miginiac-Maslow, M., Van Breusegem, F. and Noctor, G. (2007). Conditional oxidative stress responses in the Arabidopsis photorespiratory mutant cat2 demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H2O2-induced cell death. Plant J *52*, 640–657.

[Radi, 2004] Radi, R. (2004). Nitric oxide, oxidants, and protein tyrosine nitration. Proc Natl Acad Sci U S A *101*, 4003–4008.

[Ray et al., 2012] Ray, M., Mishra, P., Das, P. and Sabat, S. C. (2012). Expression and purification of soluble bio-active rice plant catalase-A from recombinant Escherichia coli. J Biotechnol *157*, 12–19.

[Reichheld et al., 2002] Reichheld, J.-P., Mestres-Ortega, D., Laloi, C. and Meyer, Y. (2002). The multigenic family of thioredoxin h in Arabidopsis thaliana: specific expression and stress response. Plant Physiology and Biochemistry *40*, 685–690.

[Ren et al., 2008a] Ren, C.-M., Zhu, Q., Gao, B.-D., Ke, S.-Y., Yu, W.-C., Xie, D.-X. and Peng, W. (2008a). Transcription factor WRKY70 displays important but no indispensable roles in jasmonate and salicylic acid signaling. J Integr Plant Biol *50*, 630–637.

[Ren et al., 2008b] Ren, D., Liu, Y., Yang, K.-Y., Han, L., Mao, G., Glazebrook, J. and Zhang, S. (2008b). A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in Arabidopsis. Proc Natl Acad Sci U S A *105*, 5638–5643.

REFERENCES

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

[Rietz et al., 2011] Rietz, S., Stamm, A., Malonek, S., Wagner, S., Becker, D., Medina-Escobar, N., Vlot, A. C., Feys, B. J., Niefind, K. and Parker, J. E. (2011). Different roles of Enhanced Disease Susceptibility1 (EDS1) bound to and dissociated from Phytoalexin Deficient4 (PAD4) in Arabidopsis immunity. New Phytol *191*, 107–119.

[Ritsema et al., 2010] Ritsema, T., van Zanten, M., Leon-Reyes, A., Voesenek, L. A. C. J., Millenaar, F. F., Pieterse, C. M. J. and Peeters, A. J. M. (2010). Kinome profiling reveals an interaction between jasmonate, salicylate and light control of hyponastic petiole growth in Arabidopsis thaliana. PLoS One *5*, e14255.

[Rizhsky et al., 2002] Rizhsky, L., Hallak-Herr, E., Van Breusegem, F., Rachmilevitch, S., Barr, J. E., Rodermel, S., Inzé, D. and Mittler, R. (2002). Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J *32*, 329–342.

[Robatzek et al., 2007] Robatzek, S., Bittel, P., Chinchilla, D., Köchner, P., Felix, G., Shiu, S.-H. and Boller, T. (2007). Molecular identification and characterization of the tomato flagellin receptor LeFLS2, an orthologue of Arabidopsis FLS2 exhibiting characteristically different perception specificities. Plant Mol Biol *64*, 539–547.

[Robert-Seilaniantz et al., 2011a] Robert-Seilaniantz, A., Grant, M. and Jones, J. D. G. (2011a). Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol *49*, 317–343.

[Robert-Seilaniantz et al., 2011b] Robert-Seilaniantz, A., MacLean, D., Jikumaru, Y., Hill, L., Yamaguchi, S., Kamiya, Y. and Jones, J. D. G. (2011b). The microRNA miR393 re-directs secondary metabolite biosynthesis away from camalexin and towards glucosinolates. Plant J *67*, 218–231.

[Robert-Seilaniantz et al., 2007] Robert-Seilaniantz, A., Navarro, L., Bari, R. and Jones, J. D. G. (2007). Pathological hormone imbalances. Curr Opin Plant Biol *10*, 372–379.

[Robson and Vanlerberghe, 2002] Robson, C. A. and Vanlerberghe, G. C. (2002). Transgenic plant cells lacking mitochondrial alternative oxidase have increased susceptibility to mitochondria-dependent and -independent pathways of programmed cell death. Plant Physiol *129*, 1908–1920.

[Rochon et al., 2006] Rochon, A., Boyle, P., Wignes, T., Fobert, P. R. and Després, C. (2006). The coactivator function of Arabidopsis NPR1 requires the core of its BTB/POZ domain and the oxidation of C-terminal cysteines. Plant Cell *18*, 3670–3685.

[Rockel et al., 2002] 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.

[Rodriguez et al., 2010] Rodriguez, M. C. S., Petersen, M. and Mundy, J. (2010). Mitogen-activated protein kinase signaling in plants. Annu Rev Plant Biol *61*, 621–649.

[Romero-Puertas et al., 2008] Romero-Puertas, M. C., Campostrini, N., Mattè, A., Righetti, P. G., Perazzolli, M., Zolla, L., Roepstorff, P. and Delledonne, M. (2008). Proteomic analysis of S-nitrosylated proteins in Arabidopsis thaliana undergoing hypersensitive response. Proteomics *8*, 1459–1469.

[Romero-Puertas et al., 2007] Romero-Puertas, M. C., Laxa, M., Mattè, A., Zaninotto, F., Finkemeier, I., Jones, A. M. E., Perazzolli, M., Vandelle, E., Dietz, K.-J. and Delledonne, M. (2007). S-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. Plant Cell *19*, 4120–4130.

[Romero-Puertas et al., 2004] Romero-Puertas, M. C., Perazzolli, M., Zago, E. D. and Delledonne, M. (2004). Nitric oxide signalling functions in plant-pathogen interactions. Cell Microbiol *6*, 795–803.

REFERENCES

[Rozen and Skaletsky, 1999] Rozen, S. and Skaletsky, H. (1999). Primer3 on the WWW for general users and for biologist programmers. In Bioinformatics methods and protocols pp. 365–386. Springer.

[Rubbo and Radi, 2008] Rubbo, H. and Radi, R. (2008). Protein and lipid nitration: role in redox signaling and injury. Biochim Biophys Acta *1780*, 1318–1324.

[Ruegger et al., 1997] Ruegger, M., Dewey, E., Hobbie, L., Brown, D., Bernasconi, P., Turner, J., Muday, G. and Estelle, M. (1997). Reduced naphthylphthalamic acid binding in the tir3 mutant of Arabidopsis is associated with a reduction in polar auxin transport and diverse morphological defects. Plant Cell *9*, 745–757.

[Rushton et al., 2010] Rushton, P. J., Somssich, I. E., Ringler, P. and Shen, Q. J. (2010). WRKY transcription factors. Trends Plant Sci *15*, 247–258.

[Rustérucci et al., 2007] Rustérucci, C., Espunya, M. C., Díaz, M., Chabannes, M. and Martínez, M. C. (2007). S-nitrosoglutathione reductase affords protection against pathogens in Arabidopsis, both locally and systemically. Plant Physiol *143*, 1282–1292.

[Rustérucci et al., 2001] Rustérucci, C.rucci, C., Aviv, D. H., Holt, 3rd, B., Dangl, J. L. and Parker, J. E. (2001). The disease resistance signaling components EDS1 and PAD4 are essential regulators of the cell death pathway controlled by LSD1 in Arabidopsis. Plant Cell *13*, 2211–2224.

[Ryals et al., 1997] Ryals, J., Weymann, K., Lawton, K., Friedrich, L., Ellis, D., Steiner, H. Y., Johnson, J., Delaney, T. P., Jesse, T., Vos, P. and Uknes, S. (1997). The Arabidopsis NIM1 protein shows homology to the mammalian transcription factor inhibitor I kappa B. Plant Cell *9*, 425–439.

[Sadanandom et al., 2000] Sadanandom, A., Poghosyan, Z., Fairbairn, D. J. and Murphy, D. J. (2000). Differential regulation of plastidial and cytosolic isoforms of peptide methionine sulfoxide reductase in Arabidopsis. Plant Physiol *123*, 255–264.

[Sagi et al., 2004] Sagi, M., Davydov, O., Orazova, S., Yesbergenova, Z., Ophir, R., Stratmann, J. W. and Fluhr, R. (2004). Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in Lycopersicon esculentum. Plant Cell *16*, 616–628.

[Saito et al., 2006] Saito, S., Yamamoto-Katou, A., Yoshioka, H., Doke, N. and Kawakita, K. (2006). Peroxynitrite generation and tyrosine nitration in defense responses in tobacco BY-2 cells. Plant Cell Physiol *47*, 689–697.

[Sakamoto et al., 2002] Sakamoto, A., Ueda, M. and Morikawa, H. (2002). Arabidopsis glutathione-dependent formaldehyde dehydrogenase is an S-nitrosoglutathione reductase. FEBS letters *515*, 20–24.

[Sambrook et al., 2001] Sambrook, J., Russell, D. W. and Russell, D. W. (2001). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press Cold Spring Harbor New York.

[Samuel et al., 2000] Samuel, M. A., Miles, G. P. and Ellis, B. E. (2000). Ozone treatment rapidly activates MAP kinase signalling in plants. Plant J *22*, 367–376.

[Sandermann, 2000] Sandermann, Jr, H. (2000). Active oxygen species as mediators of plant immunity: three case studies. Biol Chem *381*, 649–653.

[Saraste et al., 1990] Saraste, M., Sibbald, P. R. and Wittinghofer, A. (1990). The P-loop - a common motif in ATP-and GTP-binding proteins. Trends in biochemical sciences *15*, 430–434.

[Sato et al., 2010] Sato, M., Tsuda, K., Wang, L., Coller, J., Watanabe, Y., Glazebrook, J. and Katagiri, F. (2010). Network modeling reveals prevalent negative regulatory relationships between signaling sectors in Arabidopsis immune signaling. PLoS Pathog *6*, e1001011.

[Scheel, 1998] Scheel, D. (1998). Resistance response physiology and signal transduction. Curr Opin Plant Biol *1*, 305–310.

REFERENCES

[Schenk et al., 2000] Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S. C. and Manners, J. M. (2000). Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc Natl Acad Sci U S A *97*, 11655–11660.

[Schlaeppi et al., 2008] Schlaeppi, K., Bodenhausen, N., Buchala, A., Mauch, F. and Reymond, P. (2008). The glutathione-deficient mutant pad2-1 accumulates lower amounts of glucosinolates and is more susceptible to the insect herbivore Spodoptera littoralis. Plant J *55*, 774–786.

[Schmelz et al., 2003] Schmelz, E. A., Engelberth, J., Alborn, H. T., O'Donnell, P., Sammons, M., Toshima, H. and Tumlinson, 3rd, J. H. (2003). Simultaneous analysis of phytohormones, phytotoxins, and volatile organic compounds in plants. Proc Natl Acad Sci U S A *100*, 10552–10557.

[Schopfer et al., 2002] Schopfer, P., Liszkay, A., Bechtold, M., Frahry, G. and Wagner, A. (2002). Evidence that hydroxyl radicals mediate auxin-induced extension growth. Planta *214*, 821–828.

[Seo et al., 2001] Seo, H. S., Song, J. T., Cheong, J. J., Lee, Y. H., Lee, Y. W., Hwang, I., Lee, J. S. and Choi, Y. D. (2001). Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. Proc Natl Acad Sci U S A *98*, 4788–4793.

[Shah, 2003] Shah, J. (2003). The salicylic acid loop in plant defense. Curr Opin Plant Biol *6*, 365–371.

[Shah et al., 1997] 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.

[Shan et al., 2008] Shan, L., He, P., Li, J., Heese, A., Peck, S. C., Nürnberger, T., Martin, G. B. and Sheen, J. (2008). Bacterial effectors target the common signaling partner BAK1 to disrupt multiple MAMP receptor-signaling complexes and impede plant immunity. Cell Host Microbe *4*, 17–27.

[Shao et al., 2003] Shao, F., Golstein, C., Ade, J., Stoutemyer, M., Dixon, J. E. and Innes, R. W. (2003). Cleavage of Arabidopsis PBS1 by a bacterial type III effector. Science *301*, 1230–1233.

[Shapiro and Zhang, 2001] Shapiro, A. D. and Zhang, C. (2001). The role of NDR1 in avirulence gene-directed signaling and control of programmed cell death in Arabidopsis. Plant Physiol *127*, 1089–1101.

[Sheard et al., 2010] Sheard, L. B., Tan, X., Mao, H., Withers, J., Ben-Nissan, G., Hinds, T. R., Kobayashi, Y., Hsu, F.-F., Sharon, M., Browse, J., He, S. Y., Rizo, J., Howe, G. A. and Zheng, N. (2010). Jasmonate perception by inositol-phosphate-potentiated COI1-JAZ co-receptor. Nature *468*, 400–405.

[Shirano et al., 2002] Shirano, Y., Kachroo, P., Shah, J. and Klessig, D. F. (2002). A gain-of-function mutation in an Arabidopsis Toll Interleukin1 receptor-nucleotide binding site-leucine-rich repeat type R gene triggers defense responses and results in enhanced disease resistance. Plant Cell *14*, 3149–3162.

[Shiu and Bleecker, 2001] Shiu, S. H. and Bleecker, A. B. (2001). Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proc Natl Acad Sci U S A *98*, 10763–10768.

[Shiu and Bleecker, 2003] Shiu, S. H. and Bleecker, A. B. (2003). Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in Arabidopsis. Plant Physiol *132*, 530–543.

[Simon et al., 2010] Simon, C., Langlois-Meurinne, M., Bellvert, F., Garmier, M., Didier-laurent, L., Massoud, K., Chaouch, S., Marie, A., Bodo, B., Kauffmann, S., Noctor, G. and Saindrenan, P. (2010). The differential spatial distribution of secondary metabolites in Arabidopsis leaves reacting hypersensitively to Pseudomonas syringae pv. tomato is dependent on the oxidative burst. J Exp Bot *61*, 3355–3370.

REFERENCES

[Singh et al., 1996] Singh, S. P., Wishnok, J. S., Keshive, M., Deen, W. M. and Tannenbaum, S. R. (1996). The chemistry of the S-nitrosoglutathione/glutathione system. Proc Natl Acad Sci U S A *93*, 14428–14433.

[Slaymaker et al., 2002] Slaymaker, D. H., Navarre, D. A., Clark, D., del Pozo, O., Martin, G. B. and Klessig, D. F. (2002). The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. Proc Natl Acad Sci U S A *99*, 11640–11645.

[Song et al., 2011] Song, J., Durrant, W. E., Wang, S., Yan, S., Tan, E. H. and Dong, X. (2011). DNA repair proteins are directly involved in regulation of gene expression during plant immune response. Cell Host Microbe *9*, 115–124.

[Sorhagen, 2010] Sorhagen, K. (2010). Unravelling the roles of S-nitrosothiols in plant biology. PhD thesis, The University of Edinburgh.

[Souza et al., 2000] Souza, J. M., Choi, I., Chen, Q., Weisse, M., Daikhin, E., Yudkoff, M., Obin, M., Ara, J., Horwitz, J. and Ischiropoulos, H. (2000). Proteolytic degradation of tyrosine nitrated proteins. Arch Biochem Biophys *380*, 360–366.

[Spoel and Dong, 2008] Spoel, S. H. and Dong, X. (2008). Making sense of hormone crosstalk during plant immune responses. Cell Host Microbe *3*, 348–351.

[Spoel et al., 2007] Spoel, S. H., Johnson, J. S. and Dong, X. (2007). Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. Proc Natl Acad Sci U S A *104*, 18842–18847.

[Spoel et al., 2003] Spoel, S. H., Koornneef, A., Claessens, S. M. C., Korzelius, J. P., Van Pelt, J. A., Mueller, M. J., Buchala, A. J., Métraux, J.-P., Brown, R., Kazan, K., Van Loon, L. C., Dong, X. and Pieterse, C. M. J. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell *15*, 760–770.

[Spoel and Loake, 2011] Spoel, S. H. and Loake, G. J. (2011). Redox-based protein modifications: the missing link in plant immune signalling. Curr Opin Plant Biol *14*, 358–364.

[Spoel et al., 2009] Spoel, S. H., Mou, Z., Tada, Y., Spivey, N. W., Genschik, P. and Dong, X. (2009). Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. Cell *137*, 860–872.

[Spoel et al., 2010] Spoel, S. H., Tada, Y. and Loake, G. J. (2010). Post-translational protein modification as a tool for transcription reprogramming. New Phytol *186*, 333–339.

[Srivastava et al., 2009] Srivastava, N., Gonugunta, V. K., Puli, M. R. and Raghavendra, A. S. (2009). Nitric oxide production occurs downstream of reactive oxygen species in guard cells during stomatal closure induced by chitosan in abaxial epidermis of Pisum sativum. Planta *229*, 757–765.

[Staab et al., 2008] Staab, C., Hellgren, M. and Hoog, J. (2008). Dual functions of alcohol dehydrogenase 3: implications with focus on formaldehyde dehydrogenase and S-nitrosoglutathione reductase activities. Cell. Mol. Life Sci *65*, 3950–3960.

[Stamler et al., 2001] Stamler, J. S., Lamas, S. and Fang, F. C. (2001). Nitrosylation. the prototypic redox-based signaling mechanism. Cell *106*, 675.

[Stamler et al., 1992] Stamler, J. S., Singel, D. J. and Loscalzo, J. (1992). Biochemistry of nitric oxide and its redox-activated forms. Science *258*, 1898–1902.

[Staswick and Tiryaki, 2004] Staswick, P. E. and Tiryaki, I. (2004). The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. Plant Cell *16*, 2117–2127.

[Steber et al., 1998] Steber, C. M., Cooney, S. E. and McCourt, P. (1998). Isolation of the GA-response mutant sly1 as a suppressor of ABI1-1 in Arabidopsis thaliana. Genetics *149*, 509–521.

REFERENCES

[Straus et al., 2010] Straus, M. R., Rietz, S., Ver Loren van Themaat, E., Bartsch, M. and Parker, J. E. (2010). Salicylic acid antagonism of EDS1-driven cell death is important for immune and oxidative stress responses in Arabidopsis. Plant J *62*, 628–640.

[Sun et al., 2012] Sun, A., Nie, S. and Xing, D. (2012). Nitric oxide-mediated maintenance of redox homeostasis contributes to NPR1-dependent plant innate immunity triggered by lipopolysaccharides. Plant Physiol *160*, 1081–1096.

[Sun, 2011] Sun, T.-P. (2011). The molecular mechanism and evolution of the GA-GID1-DELLA signaling module in plants. Curr Biol *21*, R338–R345.

[Tada et al., 2008] Tada, Y., Spoel, S. H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J. and Dong, X. (2008). Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. Science *321*, 952–956.

[Takahashi et al., 2003] Takahashi, A., Casais, C., Ichimura, K. and Shirasu, K. (2003). HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis. Proc Natl Acad Sci U S A *100*, 11777–11782.

[Takai et al., 2008] Takai, R., Isogai, A., Takayama, S. and Che, F.-S. (2008). Analysis of flagellin perception mediated by flg22 receptor OsFLS2 in rice. Mol Plant Microbe Interact *21*, 1635–1642.

[Tao et al., 2003] Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H.-S., Han, B., Zhu, T., Zou, G. and Katagiri, F. (2003). Quantitative nature of Arabidopsis responses during compatible and incompatible interactions with the bacterial pathogen Pseudomonas syringae. Plant Cell *15*, 317–330.

[Terrile et al., 2012] Terrile, M. C., París, R., Calderón-Villalobos, L. I. A., Iglesias, M. J., Lamattina, L., Estelle, M. and Casalongué, C. A. (2012). Nitric oxide influences auxin signaling through S-nitrosylation of the Arabidopsis TRANSPORT INHIBITOR RESPONSE 1 auxin receptor. Plant J *70*, 492–500.

[Thines et al., 2007] Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S. Y., Howe, G. A. and Browse, J. (2007). JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature *448*, 661–665.

[Thordal-Christensen et al., 1997] Thordal-Christensen, H., Zhang, Z., Wei, Y. and Collinge, D. B. (1997). Subcellular localization of H2O2 in plants. H2O2 accumulation in papillae and hypersensitive response during the barley—powdery mildew interaction. The Plant Journal *11*, 1187–1194.

[Tognetti et al., 2012] Tognetti, V. B., Mühlenbock, P. and Van Breusegem, F. (2012). Stress homeostasis - the redox and auxin perspective. Plant Cell Environ *35*, 321–333.

[Tognetti et al., 2010] Tognetti, V. B., Van Aken, O., Morreel, K., Vandenbroucke, K., van de Cotte, B., De Clercq, I., Chiwocha, S., Fenske, R., Prinsen, E., Boerjan, W., Genty, B., Stubbs, K. A., Inzé, D. and Van Breusegem, F. (2010). Perturbation of indole-3-butyric acid homeostasis by the UDP-glucosyltransferase UGT74E2 modulates Arabidopsis architecture and water stress tolerance. Plant Cell *22*, 2660–2679.

[Ton et al., 2009] Ton, J., Flors, V. and Mauch-Mani, B. (2009). The multifaceted role of ABA in disease resistance. Trends Plant Sci *14*, 310–317.

[Tornero et al., 2002] Tornero, P., Merritt, P., Sadanandom, A., Shirasu, K., Innes, R. W. and Dangl, J. L. (2002). RAR1 and NDR1 contribute quantitatively to disease resistance in Arabidopsis, and their relative contributions are dependent on the R gene assayed. Plant Cell *14*, 1005–1015.

[Torres, 2010] Torres, M. A. (2010). ROS in biotic interactions. Physiol Plant *138*, 414–429.

[Torres et al., 2002] Torres, M. A., Dangl, J. L. and Jones, J. D. G. (2002). Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci U S A *99*, 517–522.

REFERENCES

[Torres et al., 2005] Torres, M. A., Jones, J. D. G. and Dangl, J. L. (2005). Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in Arabidopsis thaliana. Nat Genet *37*, 1130–1134.

[Torres et al., 2006] Torres, M. A., Jones, J. D. G. and Dangl, J. L. (2006). Reactive oxygen species signaling in response to pathogens. Plant Physiol *141*, 373–378.

[Truman et al., 2006] Truman, W., de Zabala, M. T. and Grant, M. (2006). Type III effectors orchestrate a complex interplay between transcriptional networks to modify basal defence responses during pathogenesis and resistance. Plant J *46*, 14–33.

[Truman et al., 2010] Truman, W. M., Bennett, M. H., Turnbull, C. G. N. and Grant, M. R. (2010). Arabidopsis auxin mutants are compromised in systemic acquired resistance and exhibit aberrant accumulation of various indolic compounds. Plant Physiol *152*, 1562–1573.

[Tsuda et al., 2009] Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J. and Katagiri, F. (2009). Network properties of robust immunity in plants. PLoS Genet *5*, e1000772.

[Tudzynski and Sharon, 2002] Tudzynski, B. and Sharon, A. (2002). Biosynthesis, biological role and application of fungal phytohormones. The mycota *10*, 183–211.

[Ueda et al., 2003] Ueda, M., Kinoshita, H., Maeda, S.-I., Zou, W. and Tanaka, A. (2003). Structure-function study of the amino-terminal stretch of the catalase subunit molecule in oligomerization, heme binding, and activity expression. Appl Microbiol Biotechnol *61*, 488–494.

[Uppalapati et al., 2007] Uppalapati, S. R., Ishiga, Y., Wangdi, T., Kunkel, B. N., Anand, A., Mysore, K. S. and Bender, C. L. (2007). The phytotoxin coronatine contributes to pathogen fitness and is required for suppression of salicylic acid accumulation in tomato inoculated with Pseudomonas syringae pv. tomato DC3000. Mol Plant Microbe Interact *20*, 955–965.

[Uquillas et al., 2004] Uquillas, C., Letelier, I., Blanco, F., Jordana, X. and Holuigue, L. (2004). NPR1-independent activation of immediate early salicylic acid-responsive genes in Arabidopsis. Mol Plant Microbe Interact *17*, 34–42.

[Ursini et al., 1995] Ursini, F., Maiorino, M., Brigelius-Flohé, R., Aumann, K. D., Roveri, A., Schomburg, D. and Flohé, L. (1995). Diversity of glutathione peroxidases. Methods Enzymol *252*, 38–53.

[Valls et al., 2006] Valls, M., Genin, S. and Boucher, C. (2006). Integrated regulation of the type III secretion system and other virulence determinants in Ralstonia solanacearum. PLoS Pathog *2*, e82.

[Van Breusegem et al., 2001] Van Breusegem, F., Vranová, E., Dat, J. F. and Inzé, D. (2001). The role of active oxygen species in plant signal transduction. Plant Science *161*, 405–414.

[Van der Biezen and Jones, 1998] Van der Biezen, E. A. and Jones, J. D. (1998). Plant disease-resistance proteins and the gene-for-gene concept. Trends Biochem Sci *23*, 454–456.

[Van der Ent et al., 2009] Van der Ent, S., Van Wees, S. C. M. and Pieterse, C. M. J. (2009). Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry *70*, 1581–1588.

[van Loon et al., 2006a] van Loon, L. C., Geraats, B. P. J. and Linthorst, H. J. M. (2006a). Ethylene as a modulator of disease resistance in plants. Trends Plant Sci *11*, 184–191.

[van Loon et al., 2006b] van Loon, L. C., Rep, M. and Pieterse, C. M. J. (2006b). Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol *44*, 135–162.

[van Wees et al., 2000] van Wees, S. C., de Swart, E. A., van Pelt, J. A., van Loon, L. C. and Pieterse, C. M. (2000). Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in Arabidopsis thaliana. Proc Natl Acad Sci U S A *97*, 8711–8716.

REFERENCES

[Van Wees et al., 2008] Van Wees, S. C. M., Van der Ent, S. and Pieterse, C. M. J. (2008). Plant immune responses triggered by beneficial microbes. Curr Opin Plant Biol *11*, 443–448.

[Verberne et al., 2003] Verberne, M. C., Hoekstra, J., Bol, J. F. and Linthorst, H. J. M. (2003). Signaling of systemic acquired resistance in tobacco depends on ethylene perception. Plant J *35*, 27–32.

[Verberne et al., 2000] Verberne, M. C., Verpoorte, R., Bol, J. F., Mercado-Blanco, J. and Linthorst, H. J. (2000). Overproduction of salicylic acid in plants by bacterial transgenes enhances pathogen resistance. Nat Biotechnol *18*, 779–783.

[Verhage et al., 2010] Verhage, A., van Wees, S. C. M. and Pieterse, C. M. J. (2010). Plant immunity: it's the hormones talking, but what do they say? Plant Physiol *154*, 536–540.

[Verslues et al., 2007] Verslues, P. E., Batelli, G., Grillo, S., Agius, F., Kim, Y.-S., Zhu, J., Agarwal, M., Katiyar-Agarwal, S. and Zhu, J.-K. (2007). Interaction of SOS2 with nucleoside diphosphate kinase 2 and catalases reveals a point of connection between salt stress and H2O2 signaling in Arabidopsis thaliana. Mol Cell Biol *27*, 7771–7780.

[Vieira Dos Santos and Rey, 2006] Vieira Dos Santos, C. and Rey, P. (2006). Plant thioredoxins are key actors in the oxidative stress response. Trends Plant Sci *11*, 329–334.

[Vierstra, 2003] Vierstra, R. D. (2003). The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. Trends Plant Sci *8*, 135–142.

[Vlot et al., 2009] Vlot, A. C., Dempsey, D. A. and Klessig, D. F. (2009). Salicylic Acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol *47*, 177–206.

[von Dahl and Baldwin, 2007] von Dahl, C. C. and Baldwin, I. T. (2007). Deciphering the role of ethylene in plant–herbivore interactions. Journal of Plant Growth Regulation *26*, 201–209.

[Vranová et al., 2002] Vranová, E., Atichartpongkul, S., Villarroel, R., Van Montagu, M., Inzé, D. and Van Camp, W. (2002). Comprehensive analysis of gene expression in Nicotiana tabacum leaves acclimated to oxidative stress. Proc Natl Acad Sci U S A *99*, 10870–10875.

[Wagner et al., 2004] Wagner, D., Przybyla, D., Op den Camp, R., Kim, C., Landgraf, F., Lee, K. P., Würsch, M., Laloi, C., Nater, M., Hideg, E. and Apel, K. (2004). The genetic basis of singlet oxygen-induced stress responses of Arabidopsis thaliana. Science *306*, 1183–1185.

[Walters and McRoberts, 2006] Walters, D. R. and McRoberts, N. (2006). Plants and biotrophs: a pivotal role for cytokinins? Trends Plant Sci *11*, 581–586.

[Wang et al., 2006] Wang, D., Amornsiripanitch, N. and Dong, X. (2006). A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. PLoS Pathog *2*, e123.

[Wang et al., 2007] Wang, D., Pajerowska-Mukhtar, K., Culler, A. H. and Dong, X. (2007). Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr Biol *17*, 1784–1790.

[Wang et al., 2005] Wang, D., Weaver, N. D., Kesarwani, M. and Dong, X. (2005). Induction of protein secretory pathway is required for systemic acquired resistance. Science *308*, 1036–1040.

[Wang et al., 2007] Wang, H., Ngwenyama, N., Liu, Y., Walker, J. C. and Zhang, S. (2007). Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. Plant Cell *19*, 63–73.

[Wang et al., 2010] Wang, S., Durrant, W. E., Song, J., Spivey, N. W. and Dong, X. (2010). Arabidopsis BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses. Proc Natl Acad Sci U S A *107*, 22716–22721.

[Wang et al., 2013] Wang, Y., Lin, A., Loake, G. J. and Chu, C. (2013). H2O2-induced leaf cell death and the crosstalk of reactive nitric/oxygen species. J Integr Plant Biol *55*, 202–208.

REFERENCES

[Wang et al., 2006] Wang, Y., Yun, B.-W., Kwon, E., Hong, J. K., Yoon, J. and Loake, G. J. (2006). S-nitrosylation: an emerging redox-based post-translational modification in plants. J Exp Bot *57*, 1777–1784.

[Wang et al., 2009] Wang, Y.-Q., Feechan, A., Yun, B.-W., Shafiei, R., Hofmann, A., Taylor, P., Xue, P., Yang, F.-Q., Xie, Z.-S., Pallas, J. A., Chu, C.-C. and Loake, G. J. (2009). S-nitrosylation of AtSABP3 antagonizes the expression of plant immunity. J Biol Chem *284*, 2131–2137.

[Wasternack, 2007] Wasternack, C. (2007). Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot *100*, 681–697.

[Weech et al., 2008] Weech, M.-H., Chapleau, M., Pan, L., Ide, C. and Bede, J. C. (2008). Caterpillar saliva interferes with induced Arabidopsis thaliana defence responses via the systemic acquired resistance pathway. J Exp Bot *59*, 2437–2448.

[Weigel et al., 2001] Weigel, R. R., Bäuscher, C., Pfitzner, A. J. and Pfitzner, U. M. (2001). NIMIN-1, NIMIN-2 and NIMIN-3, members of a novel family of proteins from Arabidopsis that interact with NPR1/NIM1, a key regulator of systemic acquired resistance in plants. Plant Mol Biol *46*, 143–160.

[Weigel et al., 2005] Weigel, R. R., Pfitzner, U. M. and Gatz, C. (2005). Interaction of NIMIN1 with NPR1 modulates PR gene expression in Arabidopsis. Plant Cell *17*, 1279–1291.

[Wendehenne et al., 2004] Wendehenne, D., Durner, J. and Klessig, D. F. (2004). Nitric oxide: a new player in plant signalling and defence responses. Curr Opin Plant Biol *7*, 449–455.

[Wiermer et al., 2005] Wiermer, M., Feys, B. J. and Parker, J. E. (2005). Plant immunity: the EDS1 regulatory node. Curr Opin Plant Biol *8*, 383–389.

[Wildermuth et al., 2001] Wildermuth, M. C., Dewdney, J., Wu, G. and Ausubel, F. M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature *414*, 562–565.

[Wilkinson and Crawford, 1991] Wilkinson, J. Q. and Crawford, N. M. (1991). Identification of the Arabidopsis CHL3 gene as the nitrate reductase structural gene NIA2. Plant Cell *3*, 461–471.

[Willekens et al., 1997] Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inzé, D. and Van Camp, W. (1997). Catalase is a sink for H2O2 and is indispensable for stress defence in C3 plants. EMBO J *16*, 4806–4816.

[Willekens et al., 1995] Willekens, H., Inzé, D., Van Montagu, M. and Van Camp, W. (1995). Catalases in plants. Molecular Breeding *1*, 207–228.

[Winter et al., 2007] Winter, D., Vinegar, B., Nahal, H., Ammar, R., Wilson, G. V. and Provart, N. J. (2007). An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. PLoS One *2*, e718.

[Wirthmueller et al., 2007] Wirthmueller, L., Zhang, Y., Jones, J. D. G. and Parker, J. E. (2007). Nuclear accumulation of the Arabidopsis immune receptor RPS4 is necessary for triggering EDS1-dependent defense. Curr Biol *17*, 2023–2029.

[Wolfe et al., 2000] Wolfe, J., Hutcheon, C. J., Higgins, V. J. and Cameron, R. K. (2000). A functional gene-for-gene interaction is required for the production of an oxidative burst in response to infection with avirulent Pseudomonas syringae pv. tomato in Arabidopsis thaliana. Physiological and molecular plant pathology *56*, 253–261.

[Wood et al., 2003] Wood, Z. A., Schröder, E., Robin Harris, J. and Poole, L. B. (2003). Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci *28*, 32–40.

[Woodward and Bartel, 2005] Woodward, A. W. and Bartel, B. (2005). Auxin: regulation, action, and interaction. Ann Bot *95*, 707–735.

REFERENCES

[Wu et al., 2012] Wu, Y., Zhang, D., Chu, J. Y., Boyle, P., Wang, Y., Brindle, I. D., De Luca, V. and Després, C. (2012). The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep *1*, 639–647.

[Xiao et al., 2003] Xiao, S., Brown, S., Patrick, E., Brearley, C. and Turner, J. G. (2003). Enhanced transcription of the Arabidopsis disease resistance genes RPW8.1 and RPW8.2 via a salicylic acid-dependent amplification circuit is required for hypersensitive cell death. Plant Cell *15*, 33–45.

[Xie et al., 1998] Xie, D. X., Feys, B. F., James, S., Nieto-Rostro, M. and Turner, J. G. (1998). COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science *280*, 1091–1094.

[Xu et al., 2002] Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W. L., Ma, H., Peng, W., Huang, D. and Xie, D. (2002). The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. Plant Cell *14*, 1919–1935.

[Xu et al., 2006] Xu, X., Chen, C., Fan, B. and Chen, Z. (2006). Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors. Plant Cell *18*, 1310–1326.

[Yamamoto-Katou et al., 2006] Yamamoto-Katou, A., Katou, S., Yoshioka, H., Doke, N. and Kawakita, K. (2006). Nitrate reductase is responsible for elicitin-induced nitric oxide production in Nicotiana benthamiana. Plant Cell Physiol *47*, 726–735.

[Yamasaki and Sakihama, 2000] 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.

[Yamazaki et al., 2004] Yamazaki, D., Motohashi, K., Kasama, T., Hara, Y. and Hisabori, T. (2004). Target proteins of the cytosolic thioredoxins in Arabidopsis thaliana. Plant Cell Physiol *45*, 18–27.

[Yan et al., 2009] Yan, J., Zhang, C., Gu, M., Bai, Z., Zhang, W., Qi, T., Cheng, Z., Peng, W., Luo, H., Nan, F., Wang, Z. and Xie, D. (2009). The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell *21*, 2220–2236.

[Yang and Poovaiah, 2002] Yang, T. and Poovaiah, B. W. (2002). Hydrogen peroxide homeostasis: activation of plant catalase by calcium/calmodulin. Proc Natl Acad Sci U S A *99*, 4097–4102.

[Yasuda et al., 2008] Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., Maruyama-Nakashita, A., Kudo, T., Shinozaki, K., Yoshida, S. and Nakashita, H. (2008). Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in Arabidopsis. Plant Cell *20*, 1678–1692.

[Yu et al., 1998] 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.

[Yu et al., 2012] Yu, M., Yun, B.-W., Spoel, S. H. and Loake, G. J. (2012). A sleigh ride through the SNO: regulation of plant immune function by protein S-nitrosylation. Curr Opin Plant Biol *15*, 424–430.

[Yun et al., 2011] Yun, B.-W., Feechan, A., Yin, M., Saidi, N. B. B., Le Bihan, T., Yu, M., Moore, J. W., Kang, J.-G., Kwon, E., Spoel, S. H., Pallas, J. A. and Loake, G. J. (2011). S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature *478*, 264–268.

[Yun et al., 2012] Yun, B.-W., Spoel, S. H. and Loake, G. J. (2012). Synthesis of and signalling by small, redox active molecules in the plant immune response. Biochim Biophys Acta *1820*, 770–776.

[Zander et al., 2012] Zander, M., Chen, S., Imkampe, J., Thurow, C. and Gatz, C. (2012). Repression of the Arabidopsis thaliana jasmonic acid/ethylene-induced defense pathway by TGA-interacting glutaredoxins depends on their C-terminal ALWL motif. Mol Plant *5*, 831–840.

REFERENCES

[Zander et al., 2010] Zander, M., La Camera, S., Lamotte, O., Métraux, J.-P. and Gatz, C. (2010). Arabidopsis thaliana class-II TGA transcription factors are essential activators of jasmonic acid/ethylene-induced defense responses. Plant J *61*, 200–210.

[Zaninotto et al., 2006] Zaninotto, F., La Camera, S., Polverari, A. and Delledonne, M. (2006). Cross talk between reactive nitrogen and oxygen species during the hypersensitive disease resistance response. Plant Physiol *141*, 379–383.

[Zarate et al., 2007] Zarate, S. I., Kempema, L. A. and Walling, L. L. (2007). Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol *143*, 866–875.

[Zeidler et al., 2004] Zeidler, D., Zähringer, U., Gerber, I., Dubery, I., Hartung, T., Bors, W., Hutzler, P. and Durner, J. (2004). Innate immunity in Arabidopsis thaliana: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc Natl Acad Sci U S A *101*, 15811–15816.

[Zemojtel et al., 2006] Zemojtel, T., Fröhlich, A., Palmieri, M. C., Kolanczyk, M., Mikula, I., Wyrwicz, L. S., Wanker, E. E., Mundlos, S., Vingron, M., Martasek, P. and Durner, J. (2006). Plant nitric oxide synthase: a never-ending story? Trends Plant Sci *11*, 524–5; author reply 526–8.

[Zentgraf, 2009] Zentgraf, U. (2009). Catalase regulation during leaf senescence of Arabidopsis. SEB Exp Biol Ser *62*, 187–202.

[Zhang and Shapiro, 2002] Zhang, C. and Shapiro, A. D. (2002). Two pathways act in an additive rather than obligatorily synergistic fashion to induce systemic acquired resistance and PR gene expression. BMC plant biology *2*, 9.

[Zhang et al., 2007] Zhang, J., Shao, F., Li, Y., Cui, H., Chen, L., Li, H., Zou, Y., Long, C., Lan, L., Chai, J., Chen, S., Tang, X. and Zhou, J.-M. (2007). A Pseudomonas syringae effector inactivates MAPKs to suppress PAMP-induced immunity in plants. Cell Host Microbe *1*, 175–185.

[Zhang et al., 2010] Zhang, X., Chen, S. and Mou, Z. (2010). Nuclear localization of NPR1 is required for regulation of salicylate tolerance, isochorismate synthase 1 expression and salicylate accumulation in Arabidopsis. J Plant Physiol *167*, 144–148.

[Zhang et al., 2007a] Zhang, X., Dai, Y., Xiong, Y., DeFraia, C., Li, J., Dong, X. and Mou, Z. (2007a). Overexpression of Arabidopsis MAP kinase kinase 7 leads to activation of plant basal and systemic acquired resistance. Plant J *52*, 1066–1079.

[Zhang et al., 2007b] Zhang, Y., Glazebrook, J. and Li, X. (2007b). Identification of components in disease-resistance signaling in Arabidopsis by map-based cloning. Methods Mol Biol *354*, 69–78.

[Zhang et al., 2003] Zhang, Y., Tessaro, M. J., Lassner, M. and Li, X. (2003). Knockout analysis of Arabidopsis transcription factors TGA2, TGA5, and TGA6 reveals their redundant and essential roles in systemic acquired resistance. Plant Cell *15*, 2647–2653.

[Zhong and McClung, 1996] Zhong, H. H. and McClung, C. R. (1996). The circadian clock gates expression of two Arabidopsis catalase genes to distinct and opposite circadian phases. Mol Gen Genet *251*, 196–203.

[Zhou et al., 1998] Zhou, N., Tootle, T. L., Tsui, F., Klessig, D. F. and Glazebrook, J. (1998). PAD4 functions upstream from salicylic acid to control defense responses in Arabidopsis. Plant Cell *10*, 1021–1030.

[Zhu et al., 2011] Zhu, S., Jeong, R.-D., Venugopal, S. C., Lapchyk, L., Navarre, D., Kachroo, A. and Kachroo, P. (2011). SAG101 forms a ternary complex with EDS1 and PAD4 and is required for resistance signaling against turnip crinkle virus. PLoS Pathog *7*, e1002318.

[Zhu et al., 2010] Zhu, Z., Xu, F., Zhang, Y., Cheng, Y. T., Wiermer, M., Li, X. and Zhang, Y. (2010). Arabidopsis resistance protein SNC1 activates immune responses through association with a transcriptional corepressor. Proc Natl Acad Sci U S A *107*, 13960–13965.

REFERENCES

[Zimmermann et al., 2006] Zimmermann, P., Heinlein, C., Orendi, G. and Zentgraf, U. (2006). Senescence-specific regulation of catalases in Arabidopsis thaliana (L.) Heynh. Plant Cell Environ *29*, 1049–1060.

[Zipfel, 2009] Zipfel, C. (2009). Early molecular events in PAMP-triggered immunity. Curr Opin Plant Biol *12*, 414–420.

[Zipfel et al., 2006] Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T. and Felix, G. (2006). Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts Agrobacterium-mediated transformation. Cell *125*, 749–760.

[Zipfel et al., 2004] Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E. J., Jones, J. D. G., Felix, G. and Boller, T. (2004). Bacterial disease resistance in Arabidopsis through flagellin perception. Nature *428*, 764–767.

Appendix I

Markers used for rough mapping of *spl7* and *spl8*

Marker	Location (Chr/Mb)	Primer name	Sequence
F19G10	1/8	F19G10 F	ATGTCACCGTGAACGACATC
		F19G10 R	TGCGAGTTAAGACCTAGGAG
T2E12	1/25.3	T2E12 R	CGACTAGCCAGTCCGATACA
		T2E12 F	CGTTTTGGGAGCCACGTTTC
F2G1	2/9.2	F2G1 F	CGTCGTCGGAAGTTTCAGAG
		F2G1 R	GAATAAGAAGAACACATGCGTC
T8O18	2/12.3	T8O18 F	GATATGGATGTAACGACCCAA
		T8O18 R	CAGCTTCGAGTGGATTCTAC
MIE1	3/5	MIE1 F	CTAAGTTCTTCCACCATCTG
		MIE1 R	CAAGGAGCATCTAGCCAGAG
F24B22	3/20	F24B22 F	CTGGGAACAAAGGTGTCATC
		F24B22 R	CAAGGTCTCCAGAACACAAAC
T4C9	4/6.5	T4C9 F	CAAAGGTTTCGTGTCGGAGC
		T4C9 R	CGTTGACGGGATACTCGGTG
T13J8	4/12.9	T13J8 F	ATGTTCCCAGGCTCCTTCCA
		T13J8 R	GAGATGTGGGACAAGTGACC
MYJ24	5/7.8	MYJ24 F	CTAATCCCAAGCTGAATCAC
		MYJ24 R	TGACAGAGAATCCGACTGTG
K11J9	5/24.75	K11J9 F	TACGAGCATGGTCTTGGCTA
		K11J9 R	ACTCCTCGTGTTTGGCTGAC

Appendix II

SSLP and InDel markers used for fine mapping

Marker	Type	Location (Chr/Mb)	Primer name	Sequence
F21B7	SSLP	1/0.92	F21B7-1334 F	CACGATATGATCAAGCTTTAACG
			F21B7-1334 R	TGACTACATGGAGATTATGGCC
Nga63	SSLP	1/3.55	Nga 63 F	ACCCAAGTGATCGCCACC
			Nga 63 R	AACCAAGGCACAGAAGCG
F9L1	InDel	1/5.25	F9L1 F	CACAAACCCTTCACCTCCAT
			F9L1 R	GCAGTTGCCTAAAGGCTGAG
SRP54A	SSLP	1/5.27	SRP54A F	AAAAGGAACCCTACCAAAAACA
			SRP54A R	TGAATTATGGAATCAATGTTCG
T24D18	InDel	1/5.48	T24D18 F	CCTCTTGGCATGGAAACATT
			T24D18 R	TGAGCATTGTGTAGATCATTTGC
F19K19	InDel	1/5.71	F19K19 F	TCCCCCAAAGGGATATAAGC
			F19K19 R	TGCCAATTGAAGCAGAAGAA
T13M22	InDel	1/5.92	T13M22 F	TGCCCTTTGACCTAGCATCT
			T13M22 R	CTTCACGGTGGAACTTTGGT
T20H2	InDel	1/6.9	T20H2 F	GCTTACCAGAAGCATCCTCAA
			T20H2 R	CATGGGGACATGACATTGAA
T22I11	InDel	1/7.37	T22I11 F	CCAAGTTCCCATGCTGAGTT
			T22I11 R	AATTGCAGGTCCTGATGACA
AthSO392	SSLP	1/10.86	AthSO392 F	GTTGATCGCAGCTTGATAAGC
			AthSO392 R	TTTGGAGTTAGACACGGATCTG

Appendix III

SNAP markers used for fine mapping

Marker	Location (Chr/Mb)	Col-0/ *Ler*	Primer name	Sequence
F25I1	1/6.38	Col-0	F25I16_1/6.38_Col1_F F25I16_1/6.38_Col1_R	GGGAAATCAAAACAGAACTACCGAGCGT GCTTGGACTTATATTAGCAATCATCACCGTGTT
		Ler	F25I16_1/6.38_Ler1_F F25I16_1/6.38_Ler1_R	GGAAATCAAAACAGAACTACCGAGCGC GCTTGGACTTATATTAGCAATCATCACCGTGTT
T29M8	1/6.62	Col-0	T29M8_1/6.62_Col_F T29M8_1/6.62_Col_R	TGATCAAAACGGATTACAACGAGCATGAA GCCACATTGGACTCAGCTGCAGG
		Ler	T29M8_1/6.62_Ler2_F T29M8_1/6.62_Ler2_R	GGGTATGCTTTCCGATCATAATTATCGTGC TCGCGATAAAGAGTGGATGGGATCAG
F18O14	1/6.75	Col-0	F18O14_1/6.75_Col2-F F18O14_1/6.75_Col2-R	GGGTGTGCAACTTTCTTCAGTACTTTGGG CAGAACCTAAGAAGCCGAGAGGCAGG
		Ler	F18O14_1/6.75_Ler2-F F18O14_1/6.75_Ler2-R	TGGGTGTGCAACTTTCTTCAGTACTTGTGT TCGAAAACAACCATCTGGTGAAACTACTGAA
F14P1	1/6.81	Col-0	F14P1_1/6.81_Col2-F F14P1_1/6.81_Col2-R	GCTTTGCAGTCCTGACTTATACGAAGGATAAAA AGATATATGCCAAGTTCTTAAAGCTGCCACTGA
		Ler	F14P1_1/6.81_Ler2-F F14P1_1/6.81_Ler2-R	CTTTGCAGTCCTGACTTATACGAAGGATATCG AGATATATGCCAAGTTCTTAAAGCTGCCACTGA
T20H2	1/6.92	Col-0	T20H2_1/6.92_Col2-F T20H2_1/6.92_Col2-R	AATTCATTACGAATCATTCATTAGTCAACTCCAAGA TTAGCAAAAAGTGAACTTGGAATCTTGGCA
		Ler	T20H2_1/6.92_Ler2-F T20H2_1/6.92_Ler2-R	TTCATTACGAATCATTCATTAGTCAACTCCGACT TTAGCAAAAAGTGAACTTGGAATCTTGGCA
T20H2-B	1/7	Col-0	T20H2-B_1/7_Col-F T20H2-B_1/7_Col-R	GACTGCGGAACATAAGTATCTCCTACGCA CCTGTTGCTTCCTCTCTCTCCTCTTCTCAT
		Ler	T20H2-B_1/7_Ler-F T20H2-B_1/7_Ler-R	GACTGCGGAACATAAGTATCTCCTACCCG CCTGTTGCTTCCTCTCTCTCCTCTTCTCAT
F14O10	1/7.03	Col-0	F14O10_1/7.03_Col-F F14O10_1/7.03_Col-R	AGGTCTTATATGTTCTGTTGTGTCTGAAGCTTGTG ATCTATTGTCCAAGCACAACACATGTGGGAC
		Ler	F14O10_1/7.03_Ler-F F14O10_1/7.03_Ler-R	AGGTCTTATATGTTCTGTTGTGTCTGAAGCTTCCC TATTGTCCAAGCACAACACATGTGGGACTAAA
F14O10-B	1/7.06	Col-0	F14O10-B_1/7.06_Col-F F14O10-B_1/7.06_Col-R	CATTGACATGAAACTTGTTCTGAAATCAGGTTACG CCATCATCAGTGACTTTGGATCCGAACA
		Ler	F14O10-B_1/7.06_Ler-F F14O10-B_1/7.06_Ler-R	CATTGACATGAAACTTGTTCTGAAATCAGGTGAAA CTTCCCATCATCAGTGACTTTGGATCCG
F5M15-B	1/7.09	Col-0	F5M15-B_1/7.09_Col-F F5M15-B_1/7.09_Col-R	GGACGACTACCACGTTCCTTCGCACAG ACCGCCGAGAGAAGCTTAGCCATGTC
		Ler	F5M15-B_1/7.09_Ler-F F5M15-B_1/7.09_Ler-R	GGACGACTACCACGTTCCTTCGCAGAC ACCGCCGAGAGAAGCTTAGCCATGTC
F5M15	1/7.11	Col-0	F5M15_1/7.11_Col-F F5M15_1/7.11_Col-R	AAAGCTCTGTTTATCAATGTAATGTTCGGA TTCATCGCGTAACTTGTTTATCGTCAGACA
		Ler	F5M15_1/7.11_Ler-F F5M15_1/7.11_Ler-R	AAAGCTCTGTTTATCAATGTAATGTTCGCG TTCATCGCGTAACTTGTTTATCGTCAGACA
F2D10-B	1/7.13	Col-0	F2D10-B_1/7.13_Col-F F2D10-B_1/7.13_Col-R	TCTAAGATTAGCACATGTAGCTTCTGACTATTCGCC CTACCTGATTTTCAAGCATCTCCGGTAAATGAA
		Ler	F2D10-B_1/7.13_Ler-F F2D10-B_1/7.13_Ler-R	TCTAAGATTAGCACATGTAGCTTCTGACTATTCGCT CTACCTGATTTTCAAGCATCTCCGGTAAATGA
F2D10-C	1/7.16	Col-0	F2D10-C_1/7.16_Col-F F2D10-C_1/7.16_Col-R	CGCTTCCCAATCTCCCACAATAGATCCC GACCACGCCTCCTCCTCCGCC
		Ler	F2D10-C_1/7.16_Ler-F F2D10-C_1/7.16_Ler-R	CGCTTCCCAATCTCCCACAATAGATGTG CAGTGCAGCTTGCAAAGTCCCCTGT
F2D10	1/7.23	Col-0	F2D10_1/7.23_Col1-F F2D10_1/7.23_Col1-R	TCACATTCCATTTTCCTTTCAAGTTCGTGTAA GAGGCTTCAACAAACCTTGGAGTTGGTT
		Ler	F2D10_1/7.23_Ler1-F F2D10_1/7.23_Ler1-R	CATTCCATTTTCCTTTCAAGTTCGTGGTG CAGGAGCTGGAGTGCCAAGTTACTTCTG

Appendix IV

Primers used for genotyping T-DNA lines

Line	Gene	Primer name	Sequence
atgsnor1-3	*At5g43940*	315 D11 Left border	ATATTGAACATCATACTCATTG
		315 D11 Forward	TATATAATGGTTCGACGATAT
		315 D11 Reverse	CCACCAACACTCTCAACAATC
cat1	*At1g20630*	Lb1	GCGTGGACCGCTTGCTGCAACT
		CAT1 GT F	GTAAGAGATCCAAATGCTGCG
		CAT1 GT R	ATTGAAACCGAATCCCAAGTC
cat2	*At4g35090*	LBb1	GCGTGGACCGCTTGCTGCAACT
		CAT2-LP2	TCGCATGACTGTGGTTGGTTC
		CAT2-RP2	ACCACCAACTCTGGTGCTCCT
cat3	*At1g20620*	LBb1	GCGTGGACCGCTTGCTGCAACT
		CAT3-LP	CACCTGAGTAATCAAATCTACACG
		CAT3-RP	TCAGGGATCCTCTCTCTGGTGAA
trx3	*At5g42980*	LBb1.3	ATTTTGCCGATTTCGGAAC
		TRX3 GT F	GCTGCGAGTAATCAAGTTTGC
		TRX3 GT R	ACCGACACAGAGACGAAGAAG
trx5	*At1g45145*	LBb1.3	ATTTTGCCGATTTCGGAAC
		TRX5 GT F2	GAAGCTACAAGACCACCATGC
		TRX5 GT R	TTCTCTTGTTATGTCCAGGGC

Appendix IV

Primers used for genotyping point mutation lines

Line	Gene	WT/mut	Primer name	Sequence
spl7	*At1g20620*	WT	spl7_GT_WT_F	AACCACTCTCTCAGGGATCCTCTCGC
			spl7_GT_WT_R	ATCGATCGTATAATGGTGATTGCAGTATCGTC
		spl7	spl7_GT_Mutant_F	GAACCACTCTCTCAGGGATCCTCGCTT
			spl7_GT_Mutant_R	ATCGATCGTATAATGGTGATTGCAGTATCGTC
spl8	*At1g20620*	WT	spl8_GT_WT_F	CTCTGGTGTAAAACTTGACAGCAAAACAACG
			spl8_GT_WT_R	AGCTGAGATCTTGTTCGTGAAGCGTGAT
		spl8	spl8_GT_Mutant_F	GGTGTAAAACTTGACAGCAAAACCGCA
			spl8_GT_Mutant_R	AGCTGAGATCTTGTTCGTGAAGCGTGAT
pad2	*At4g23100*	WT	pad2_WT_F1	AAGGAAAGCCAAACGGATTTCTCCG
			pad2_WT_R1	GATCCAAAGCATCTTTCTATCTTGAACACAAACATA
		pad2	pad2_mut_F1	AAGGAAAGCCAAACGGATTTCCCAA
			pad2_mut_R1	GATCCAAAGCATCTTTCTATCTTGAACACAAACATA
vtc2-1*	*At4g26850*		VTC2RTPCR LP	TCAGCTTAACGAGGGTCGTCAC
			VTC2RTPCR RP	GGCAAACACAGCAGTCTGAAAC