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"Identification of new regulators for systemic acquired resistance (SAR) in plants by an integrated proteomics approach"

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II. ABBREVIATIONS

aa	Amino acid(s)
bp	Base pairs
cfu	Colony forming units
DNA	Deoxyribonucleic acid
DC3000	*Pseudomonas syringae* pv. *tomato* DC3000 - virulent
GOI	Gene of interest
h	Hour(s)
Kb	Kilobases
kDa	Kilodalton
LB	Luria-Bertani
ml	Milliliter(s)
min	Minute(s)
mM	Millimolar
MS	Mass spectrometry
nt(s)	Nucleotide(s)
OD	Optical density
O/N	Overnight
ORF	Open reading frame
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
Pst	*Pseudomonas syringae* pv. tomato (DC3000)
RNA	Ribonucleic acid
ROI	Region of interest
rpm	Revolutions per minute
RT	Room temperature
wt	Wild type
Y2H	Yeast-Two-Hybrid
µl	Microliter(s)
µM	Micromolar
λ_{ex}	Extinction wavelength
λ_{em}	Emission wavelength

Amino acids and nucleotides are expressed according to the IUPAC code (International Union of Pure and Applied Chemistry). All base units and derived units are used following the convention of the SI-system (Système International d'unitès).

III. SUMMARY

Systemic acquired resistance (SAR) plays a fundamental role in protecting plants against various diseases. SAR is a state of heightened defense, which is activated systemically following a primary infection. Prior to SAR establishment, mobile signals are transported from the infected site through the phloem to the systemic leaves. SAR-like disease resistance can be induced in *Arabidopsis thaliana* by over expression of the bacterial effector *AvrRpm1* from a dexamethasone-inducible transgene. Thus induced resistance/SAR reduces growth of virulent bacteria in systemic, non-*AvrRpm1*-expressing tissues.

ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) encodes one of the main regulators of SA signaling and is essential for SAR signal generation and/or transmission (Jorda, Vlot, and Parker, personal communication). By comparing the protein profile of *AvrRpm1*-expressing wild type plants against similar extracts from *eds1-2* mutant plants we identified new potential SAR-inducing signals or signaling regulators. 2D-gel (Vlot *et al*., conducted at the Max Planck Institute for Plant Breeding Research, Cologne, Germany - unpublished), LC-MS/MS (high-performance liquid chromatography coupled with mass spectrometry) and ICPL analysis (Isotope-Coded Protein Labeling) led to the detection of 21 proteins, which reproducibly accumulate in the apoplast of *AvrRpm1*-expressing *Arabidopsis* in an *EDS1*-dependent manner. The new candidate SAR signaling proteins were termed *At*AED, for *Arabidopsis thaliana* Apoplastic, *EDS1*-Dependent. *AED* gene expression was analyzed after pathogen infection of *Arabidopsis* in order to evaluate which genes are regulated and therefore most interesting for further characterization.

SAR experiments with T-DNA insertion knock out (KO) mutants of two genes encoding legume lectin-like proteins, *At*AED9-1 and *At*AED9-3, revealed that both genes are required for SAR induced by *Pseudomonas syringae* pathovar *tomato* (DC3000) carrying either *AvrRpm1* or *AvrRps4*. Furthermore, gene expression analysis before and after pathogen attack showed that both genes are significantly induced after pathogen infection of *A. thaliana*. *AtAED9-3* also was induced systemically. Together, the data indicate that *At*AED9-1 and *At*AED9-3 likely play an important role in SAR.

In parallel, a new experimental approach was developed to analyze the SAR-inducing potential of proteins in tobacco in medium-high throughput. To this end, two existing techniques were combined: *Agrobacterium tumefaciens*-mediated transient protein expression and infection of tobacco with tobacco mosaic virus as a SAR read-out. By using the new approach, it was established that localized expression of a 1:1 mixture of the predicted GDSL-motif lipases *At*AED4 and *At*AED5 triggers SA-dependent SAR in tobacco.

IV. ZUSAMMENFASSUNG

Systemisch erworbene Resistenz (SAR) spielt eine fundamentale Rolle, um Pflanzen gegen verschiedenste Krankheiten zu schützen. SAR ist ein Mechanismus der pflanzlichen Abwehr, der nach einem zunächst lokal erfolgten Pathogenbefall zu einem systemischen Schutz führt. Bevor SAR aufgebaut werden kann, müssen mobile Signale von dem Ort des Pathogenbefalls über das Phloem zu den systemischen Pflanzenteilen transportiert werden. Um SAR in *Arabidopsis thaliana* zu induzieren, wurde der bakterielle Effektor *AvrRpm1* in einer transgenen Pflanze mittels Dexamethasone induziert. Die so induzierte systemisch erworbene Resistenz, führt zu einem verringerten Wachstum von virulenten Bakterien im systemischen Gewebe, welches nicht induziert wurde.

ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) codiert einen der Hauptregulatoren im Salicylsäure (SA) – Signalweg und ist von essentieller Wichtigkeit für die Erzeugung eines SAR Signals bzw. von SAR Signalen und deren Weitergabe (Jorda, Vlot, und Parker, persönliche Kommunikation). In dieser Arbeit wurde das Proteinprofil aus dem Apoplasten von *AvrRpm1* exprimierenden Wildtyp *Arabidopsis* Pflanzen mit dem von *eds1-2* Mutanten verglichen. Dabei wurden neue, potentiell SAR induzierende Signale bzw. Signalregulatoren identifiziert. In der vorliegenden Arbeit wurden drei verschiedene proteinanalytische Methoden verwendet: 2D-gel (Vlot *et al*., Max Planck Institut für Pflanzenzüchtung, Köln, Deutschland - unveröffentlicht), LC-MS/MS (high-performance liquid chromatography coupled with mass spectrometry) und ICPL analysis (Isotope-Coded Protein Labeling). Dies führte zur Detektion von 21 Proteinen, die reproduzierbar im Apoplast von *AvrRpm1*-exprimierenden *Arabidopsis* in einer *EDS1*-abhängigen Weise akkumulierten. Die neuen Kandidatenproteine wurden als *At*AED Proteine bezeichnet, was für „*A*rabidopsis *t*haliana Apoplastic, *E*DS1-Dependent" steht. Die Genexpression aller *AtAED* Kandidaten wurde nach Pathogenbefall quantitativ analysiert, um so die Regulation der Gene zu charakterisieren und anschließend die regulierten Gene weiter zu prozessieren.

SAR Experimente mit T-DNA insertion knock out (KO) Mutanten von zwei Genen, die jeweils ein Legume Lectin-like Protein kodieren, zeigten, dass beide Gene ein wichtige Rolle in SAR spielen, wenn dieses durch *Pseudomonas syringae* pathovar *tomato* (DC3000) induziert wurde. Außerdem zeigte eine Genexpressionsanalyse, die vor und nach Pathogenbefall durchgeführt wurde, dass beide Gene signifikant induziert wurden. Das bedeutet, dass *At*AED9-1 und *At*AED9-3 potentiell eine wichtige Rolle in SAR spielen.

Parallel dazu wurde eine neues Set-up entwickelt, um in einem Hochdurchsatzverfahren potentiell SAR-induzierende Proteine in Tabak zu testen. Dafür wurden zwei Methoden kombiniert: transiente Proteinexpression mittels *Agrobacterium tumefaciens* und eine anschließende Infektion von Tabak durch den Tabakmosaikvirus (TMV) als SAR Ausleseverfahren.

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Chapter 1: INTRODUCTION

The co-evolution of plants and their associated microbes has given rise to a diverse array of exchanged signals and responses (Bent 1996) (HammondKosack and Jones 1997). There are different types of mechanisms for plants to fend off attacks by plant pathogens.

The introduction of this PhD thesis focuses on the primary and the secondary immune response, giving more insights into the mode of action of phytohormones in general and in relation to plant defense. Furthermore, hormone crosstalk, especially highlighting the importance of salicylic acid (SA), will be discussed.

The mode of operation of systemic acquired resistance (SAR) in plants is introduced as well as various players in this complex signaling pathway. Finally, the experimental setup and the integrated proteomic approach used in this work are clarified, followed by a summary of the goals of this thesis.

1.1 Two layers of defense

Plants encode a multi-layered innate immune system that is actively turned on upon recognition of a pathogen. An essential prerequisite determining the mode of immunity that is used to fend off the pathogen is the mode of recognition of the pathogen by the plant. The primary immune response recognizes common features of microbial pathogens, such as flagellin, chitin, glycoproteins, and/or lipopolysaccharides. These microbial determinants are referred to as either pathogen-associated molecular patterns (**PAMPs**) or microbe-associated molecular patterns (**MAMPs**) (Mackey and McFall 2006, Schwessinger and Zipfel 2008). For the remainder of the thesis I will use the term PAMPs. PAMPs are broadly conserved among different pathogen species (Parker 2003) and contribute to general microbial fitness (Thomma, Nurnberger et al. 2011). Recognition of PAMPs is very important for further downstream signaling in the plant, but also in the animal innate immune system (Janeway and Medzhitov 2002). One example of a conserved PAMP that is acting in the plant as well as in the animal innate immune system is flagelin. Flagelin is recognized in the animal innate immune system via TLR5 (Toll-like receptor 5) (Hayashi, Smith et al. 2001) and in plants via FLS2 (a transmembrane receptor-like kinase) (Gomez-Gomez and Boller 2000).

PAMPs trigger an immune response by the activation of their cognate pattern-recognition receptors (**PRRs**) (Zipfel 2008). PRRs, in turn, initiate diverse downstream signaling events that ultimately result in the activation of a basal resistance that is called **PAMP-triggered immunity** (**PTI**) (Jones and Dangl 2006). The downstream signaling events can include the activation of MAPK (mitogen-activated protein kinase) cascades via MAPK3 or MAPK6, altered RNA metabolism via GRP7 (glycine-rich RNA-binding protein) (Fu, Guo et al. 2007), and the induction of vesicle trafficking. The plant PRRs identified so far are mostly located in the plasma membrane (Zipfel 2008) and are represented mostly by receptor protein kinases (RPKs) (Tena, Boudsocq et al. 2011) or receptor-like kinases (RLKs) (Shiu and Bleecker 2001) (Greeff, Roux et al. 2012). By contrast, PRRs in animal systems can be localized either on the cell surface or intracellularly (Takeuchi and Akira 2010). Although the number of known bacterial PAMPs recognized by plants is steadily growing, only a very few PRRs have been discovered so far (Nicaise, Roux et al. 2009) (Zipfel 2008).

Thus, a first line of defense used by plants includes PTI that can limit further colonization of the plant by the pathogen (Jones and Dangl 2006). During evolution pathogens acquired effector molecules (Gohre and Robatzek 2008) that are transported into the host cell to suppress PTI and promote virulence of the pathogen, resulting in **effector-triggered susceptibility** (**ETS**) (Jones and Dangl 2006). Effector molecules, in contrast to PAMPs, are species-, race-, or strain-specific and contribute to pathogen virulence (Thomma, Nurnberger et al. 2011). These effector molecules are secreted into the host cell via a pathogen-derived secretion system, of which the so-called type-III-protein-secretion-system (T3SS) is the most common one used. The T3SS is mostly used by bacterial pathogens to deliver effectors across the plant cell wall and plasma membrane (Alfano and Collmer 2004), but also by oomycete effectors (Fabro, Steinbrenner et al. 2011) (Bozkurt, Schornack et al. 2012). T3SS are substantially conserved among different bacteria, but the effector molecules they deliver are unique for each bacterial species (Galan and Collmer 1999). An extensive summary of T3SSs of different bacterial pathogens of animals and plants is provided by (Hueck 1998). Not only the delivery of the right effector molecules is an important function of the T3SS, but also its ability to recognize and secrete substrates in a defined order is of exceptional importance. Protein secretion must be precisely coordinated in order to successfully deliver effector proteins through the eukaryotic host cell membrane and subsequently bypass plant defense (Lara-Tejero, Kato et al. 2011).

As a consequence of the development of ETS, plants would need an 'answer' to (bacterial) effectors in order to "fight back". Accordingly, plants acquired resistance **(*R*) genes** (Belkhadir, Subramaniam et al. 2004) (Martin, Bogdanove et al. 2003) (Van der

Biezen and Jones 1998). The encoded R proteins recognize attacker-specific effectors, resulting in a secondary immune response called **effector-triggered immunity** (**ETI**). This view of the plant immune system can be referred as the **four phased "zigzag" model**, first being introduced by (Jones and Dangl 2006) (Figure 1). "Zigzag" refers to the permanent interplay between plant and pathogen, between defense and counter-defense (Chisholm, Coaker et al. 2006, Nishimura and Dangl 2010).

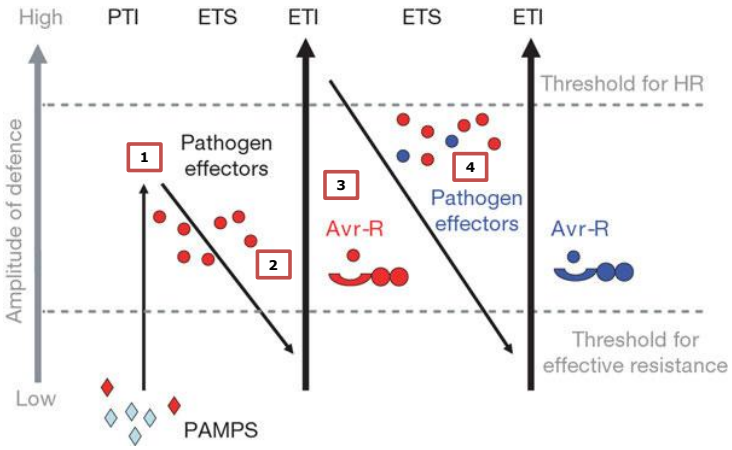


Figure 1: The four-phased "zigzag" model of plant defense.

This model illustrates the different layers of plant defense and the evolutionary fight between plant and pathogen for resistance and susceptibility including the most important players. Numbers represent the four different phases.

PTI, PAMP-triggered immunity, ETS, effector-triggered susceptibility, ETI, effector-triggered immunity, PAMPS, pathogen-associated molecular patterns, HR, hypersensitive response, Avr-R, avirulence protein/effector-Resistance protein

Figure modified from (Jones and Dangl 2006); Copyright Agreement obtained

Most *R* genes encode nucleotide-binding leucine-rich repeat (NB-LRR) proteins (Collier and Moffett 2009). These proteins are named NB-LRR, because of the presence of a conserved central nucleotide binding (NB) domain and a more variable C-terminal leucine-rich repeat (LRR) domain. With regard to their N-termini, two major groups can be distinguished within the NB-LRR proteins (Meyers, Dickerman et al. 1999). Members of the first class possess an N-terminal TIR (Toll and Interleukin-1 Receptor homology) domain, whereas the members of the second class might possess one of a number of variable N-terminal domains, some of which are predicted to form a coiled–coil (CC) structure. Studies indicate that the C-terminal LRR domain plays a role in defining the specificity of pathogen recognition (Ellis, Lawrence et al. 2007) (Shen, Zhou et al. 2003). However, also the N-terminus of the NB-LRR proteins is involved in pathogen recognition, which leads to a two-step recognition model. This two-step recognition model involves interactions of an effector with both cellular co-factors and the LRR domain of its cognate R protein, which in turn activates the molecular switch leading to disease resistance (Collier and Moffett 2009).

ETI culminates in programmed death of cells inside of and around the infection site. This is called the hypersensitive response (HR) (Mur, Kenton et al. 2008), which potently arrests pathogen growth. Formation of an HR during ETI is triggered downstream from or concomitant with other mechanisms, e.g. autophagy (Hofius, Schultz-Larsen et al. 2009), ROS (reactive oxygen species) production (Torres, Jones et al. 2006), MAPK cascade

activation (Asai, Tena et al. 2002), and hormone signaling (Jones and Dangl 2006). Figure 2 gives an overview of the basic differences in input and output between PTI and ETI.

PTI and ETI both are linked to the plant defense hormone salicylic acid (SA): a recent review (Pieterse, Leon-Reyes et al. 2009) states that PTI- and ETI-mediated pathogen recognition is associated with increased levels of SA, locally at the site of infection and also systemically in distant tissues.

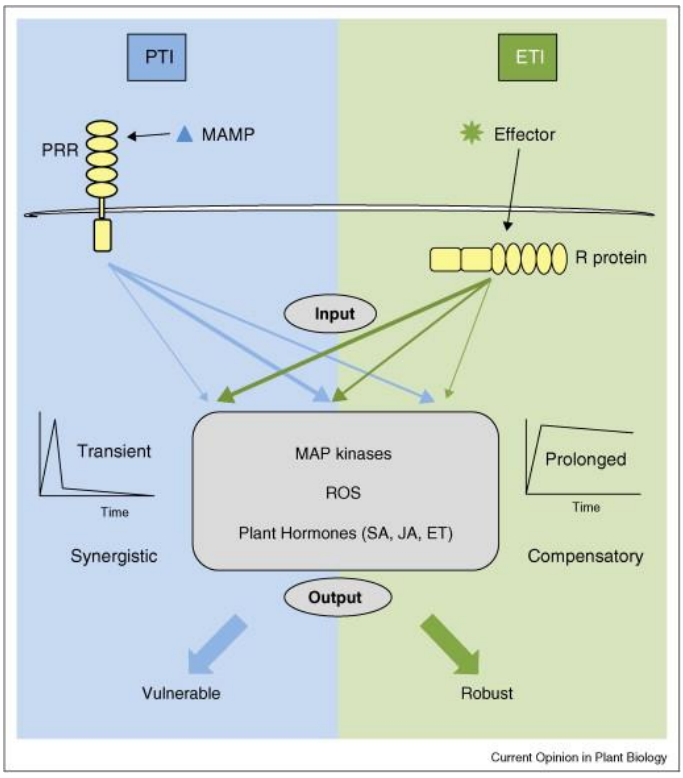


Figure 2: Comparison of PTI and ETI signaling pathway.

Common signaling machinery is used differently in PTI and ETI. A PAMP (here: MAMP) is recognized by a PRR, which leads to PTI. On the other hand, an effector molecule is recognized by an R protein leading to ETI. Via different input pathways MAP kinases are activated and ROS or plant hormones are synthesized. These responses are transient in PTI and prolonged in ETI, which results in a vulnerable output in the case of PTI and in a robust output in the case of ETI.

SA, salicylic acid, JA, jasmonic acid, ET, Ethylene, ROS, reactive oxygen species, MAP kinases, mitogen-activated protein kinases

Figure taken from (Tsuda and Katagiri 2010), Copyright Agreement obtained

Summing up, ETI and PTI are triggered by different types of pathogen molecules and are defined by different modes of pathogen recognition. During PTI PRRs recognize PAMPs, whereas during ETI the recognition of pathogen effectors is mediated by R proteins. Both detections lead to partly overlapping defense responses, but PTI can be overcome by the pathogen via effector interference with PTI signaling (Zhang, Shao et al. 2007), while ETI can only be overcome by avoiding recognition and not by attacking ETI signaling (Rosebrock, Zeng et al. 2007). The PTI defense response is based on synergistic relationships between the signaling mechanisms among the signaling sectors, which may amplify the signal, whereas compensatory relationships dominate during ETI (Tsuda, Sato et al. 2009). This “back-up” of one another may explain the robustness of the ETI answer compared to PTI.

1.2 Phytohormones - general role in plants and special role in plant defense

Phytohormones like SA, jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), auxin, gibberellins (GA) and cytokinins fulfill multiple roles. ET, ABA, auxin, GA and cytokinin are described as the five "classical" plant hormones (Kende and Zeevaart 1997). The subsequent paragraph highlights some of the general functions of JA and ET in plants followed by a closer description of their role in plant resistance.

Phytohormones are important players in signaling processes other than defense against pathogens. For instance, phytohormones play an important role in insect-specific plant reactions, where the JA pathway has emerged as the major signaling pathway at the plant-insect interface (McConn, Creelman et al. 1997). Recent findings suggest that the specificity in these interactions is mediated by different other phytohormones, with the JA pathway generally playing a dominant role (Erb, Meldau et al. 2012). Otherwise, JA is mainly known to be involved in *Arabidopsis* leaf senescence (He, Fukushige et al. 2002) and in the wound response (Stratmann 2003) (Wasternack, Stenzel et al. 2006) (Li, Li et al. 2002). The gaseous hormone ET is known to regulate multiple physiological and developmental processes in plants, such as leaf (Grbic and Bleecker 1995) and flower senescence (Reid and Wu 1992), fruit ripening (Lelievre, Latche et al. 1997), and organ abscission (Patterson and Bleecker 2004).

Furthermore, the phytohormones auxin, GA and cytokinin are among the most important regulators of *Arabidopsis* root development (Petricka, Winter et al. 2012). ABA and GA are part of a signaling network regulating seed dormancy and germination (Koornneef, Bentsink et al. 2002), also under abiotic stress, including osmotic stress (Daszkowska-Golec 2011).

As mentioned in the legend of Figure 2, certain phytohormones play an important role in the plant defense response (Bari and Jones 2009). The most important ones are **SA, JA,** and **ET** (Pieterse, Leon-Reyes et al. 2009) (GRANT AND JONES 2009). Roles of other classical phytohormones like ABA, auxins, and cytokinins, in plant defense are established, but less well known (Robert-Seilaniantz, Navarro et al. 2007).
Signaling pathways involving the hormones JA and ET are principally effective against necrotrophic pathogens, chewing insects and some phloem feeding insects, whereas those involving SA are effective against biotrophic pathogens and some phloem feeding insects (Glazebrook 2005) (Thaler, Humphrey et al. 2012). As examples, ET plays an important role in resistance against *Botrytis cinerea* in tomato (Diaz, ten Have et al. 2002) or against *Fusarium oxysporum* in *Arabidopsis* (Berrocal-Lobo and Molina 2004),

whereas JA functions in resistance to *Alternaria brassicicola* or *Botrytis cinerea* in *Arabidopsis* (Bowling, Guo et al. 1994). When JA or JA-methyl ester were applied on tomato and potato they were able to decrease growth of *Phytophtora infestans* (Cohen, Gisi et al. 1993). The role of SA in disease resistance is closely related to the research topic of this thesis and is introduced in more detail below.

1.3 Crosstalk between the phytohormones ET, JA and SA

Due to their different roles in plant development as well as in plant defense, SA, JA and ET may also affect each other. It is hypothesized that plant defense pathways interact synergistically or antagonistically to fine-tune responses according to the challenging organisms (Pieterse, Leon-Reyes et al. 2009). An example of synergism between JA and ET is the pathogen-induced expression of the plant defensin gene *PDF1.2* in *Arabidopsis,* which requires activation of the JA and ET signaling pathway for full expression (Penninckx, Thomma et al. 1998).

Cross-communication between hormone signaling pathways provides the plant with a large regulatory capacity that may tailor its defense response to different types of attackers (Pieterse, Leon-Reyes et al. 2009). To focus on some key hormones involved in plant defense the focus in this section lies on the crosstalk between SA and JA signaling. The primary mode of interaction is mutually antagonistic (Gupta, Willits et al. 2000) (Doares, Narvaez-Vasquez et al. 1995). For instance, JA insensitive *coi* mutants are impaired in JA signaling and insensitive to the bacterial effector and JA analog coronatine. Simultaneously, SA-dependent gene expression is enhanced in these mutants. At the same time SA synthesis and SA-mediated defense responses upon infection are hyper-activated (Kloek, Verbsky et al. 2001). However, synergism between SA and JA signaling has also been reported (Koornneef, Leon-Reyes et al. 2008) (Koornneef and Pieterse 2008) (Niki, Mitsuhara et al. 1998) (Vidal, deLeon et al. 1997, Niki, Mitsuhara et al. 1998). Whether crosstalk between SA and JA is synergistic or antagonistic depends at least in part on the concentrations of the hormones (Mur, Kenton et al. 2006). This means that the outcomes of JA-SA interactions could be tailored to pathogen/pest attack by the relative concentration of each hormone. Moreover, ET influences crosstalk between SA and JA. It potentiates SA-responsive *PR1* expression (De Vos, Van Zaanen et al. 2006), but also affects the outcome of the JA response: after infection by necrotrophic pathogens ET synergistically interacts with JA to trigger defense (Lorenzo, Piqueras et al. 2003) (Anderson, Badruzsaufari et al. 2004) (Pre, Atallah et al. 2008). Accordingly, (Leon-Reyes, Du et al. 2010) showed that ET counteracts the antagonistic effect of SA on JA-responsive gene expression.

The crosstalk between SA and ET is less explored than the relationship between SA and JA. Nevertheless, the limited data available suggest, that both positive (O'Donnell, Jones et al. 2001) (Schenk, Kazan et al. 2000) and negative (Lawton, Potter et al. 1994) regulatory interactions are present. The interactions and the most important players among the different plant defense mechanisms are explained in more detail in Figure 3.

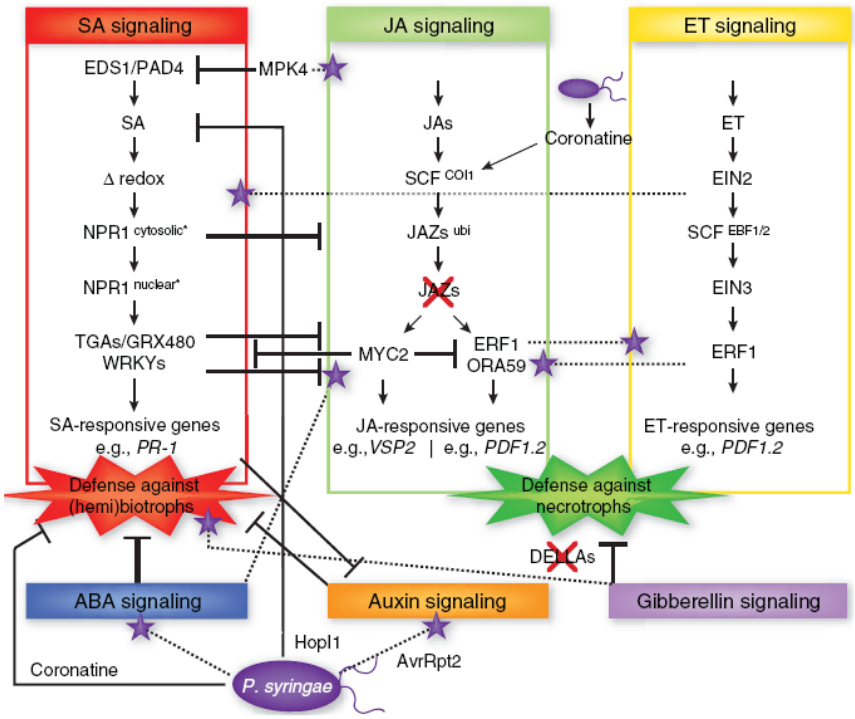


Figure 3: Networking by phytohormones in the plant immune response.

JA and ET signaling is involved in the defense response against necrotrophs, SA signaling in response to (hemi) biotrophs. The different hormones are influenced by each other either positively or negatively.

Blocked arrows: negative effect, purple stars: positive effect

Figure taken from (Pieterse, Leon-Reyes et al. 2009); Copyright Agreement obtained

Figure 3 illustrates various players involved in the SA, JA and ET hormone signaling pathways. The figure includes proteins/genes that are required to initiate different response pathways that effectively target pathogens. Moreover, the negative and positive effects among these different players are indicated, highlighting the complexity of hormonal crosstalk in plant defense. This thesis is not going into detail regarding the identity of the different regulators shown in Figure 3.

Pathogens can take advantage of the plant's ability of hormonal crosstalk by mimicking hormones of one pathway to interfere with another host immune response pathway (Spoel and Dong 2008). One example of "hormonal-mimicry" is the phytotoxin coronatine (COR) (Bender, Alarcon-Chaidez et al. 1999) produced by some *Pseudomonas syringae* strains. COR structurally resembles JA derivatives, including JA-isoleucine (Staswick 2008) and is subsequently able to interfere with SA signaling, thus promoting virulence of the COR-producing pathogen (Spoel and Dong 2008).

1.4 Salicylic acid - a major plant hormone involved in systemic acquired resistance (SAR) – synthesis and function

Salicylic acid (SA) is an important regulator of plant disease resistance (Delaney, Uknes et al. 1994) as well as of induced/systemic resistance to pathogens (Durrant and Dong 2004). Consequently, this molecule received a lot of attention in recent years. SA is a molecule with various functions (Vlot, Dempsey et al. 2009) (Raskin 1992). It influences e.g. seed germination (Rajjou, Belghazi et al. 2006), cell growth (Rate, Cuenca et al. 1999), stomatal closure in *Vicia faba* L. (Manthe, Schulz et al. 1992), responses to abiotic stresses (Yuan and Lin 2008), basal thermotolerance (Clarke, Mur et al. 2004) and nodulation in legumes (Stacey, McAlvin et al. 2006).

SA can be synthesized in plants via two distinct metabolic pathways, the isochorismate (IC) pathway and the phenylalanine ammonia-lyase (PAL) pathway. Figure 4 illustrates SA synthesis either from phenylalanine via cinnamic and/or benzoic acid (PAL pathway) or via isochorismate (IC pathway), in which case the precursors are derived from the Shikimic acid pathway.

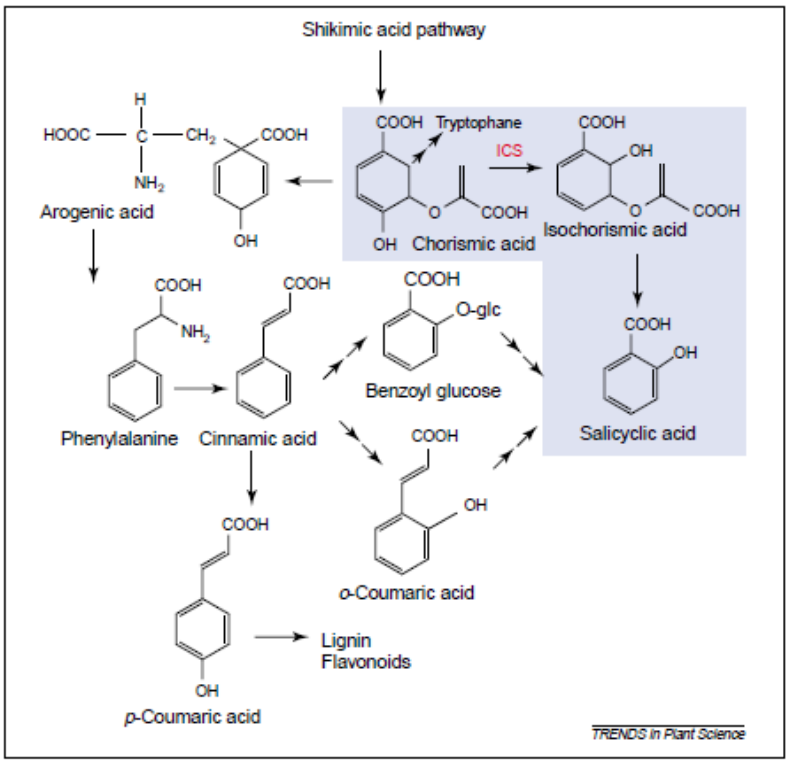


Figure 4: Pathways for synthesis of salicylic acid.

Two pathways of SA biosynthesis have been proposed in plants. Biochemical studies using isotope feeding have suggested that plants synthesize SA from cinnamic acid produced by the activity of phenyl-alanine ammonia lyase (PAL). (Chen, Zheng et al. 2009). The part highlighted in blue was originally described in bacteria and has now been shown to take place in the chloroplast of plants. In this case, SA is produced from chorismic acid via the activity of isochorismate synthase (ICS).

Figure taken from (Metraux 2002); Copyright Agreement obtained

So far it has been shown that 4 *PAL* genes in Arabidopsis encode enzymes that serve as a starting point in the phenylpropanoid pathway (Cochrane, Davin et al. 2004). The involvement of the PAL pathway in SA synthesis was shown by radio-labeling studies with pathogen-inoculated tobacco and cucumber (Yalpani, Leon et al. 1993) (Meuwly, Molders

et al. 1995), but also by *PAL* expression studies in tobacco and Arabidopsis resisting pathogen infection (Pellegrini, Rohfritsch et al. 1994) (MauchMani and Slusarenko 1996). Moreover, loss of PAL activity by using the inhibitor AIP (2-aminoindan-2-phosphonic acid) resulted in reduced SA accumulation in Arabidopsis (MauchMani and Slusarenko 1996), tobacco (Pallas, Paiva et al. 1996), and cucumber (Meuwly, Molders et al. 1995).

The rate-limiting enzyme in the biosynthesis pathway of SA in response to pathogen infection is encoded by *isochorismate synthase 1 (ICS1)* (Wildermuth, Dewdney et al. 2001), which makes the IC pathway the major source of SA in relation to pathogen defense (Durrant and Dong 2004). In addition, *isochorismate synthase 2 (ICS2)* was identified that has 83% identity at the amino acid level with *ICS1*, although it did not accumulate in the inoculated leaves of *Arabidopsis* plants following infection with the fungal biotroph *Golovinomyces orontii* (formerly called *Erysiphe orontii*) or a virulent strain of the bacterial hemi-biotroph *Pseudomonas syringae* pv. *maculicola* (*Psm)* (Wildermuth, Dewdney et al. 2001). This makes ICS1 the most important enzyme in the IC pathway. To elucidate the biochemical role and molecular distribution of ICS1, it was shown that purified mature ICS1 exhibited a specific affinity for chorismate (Strawn, Marr et al. 2007) and that ICS1 has a putative plastid transit sequence and cleavage site (Wildermuth, Dewdney et al. 2001) (Strawn, Marr et al. 2007). This is consistent with its use of plastid-synthesized chorismate as a substrate.

1.5 SAR – a systemic defense response

As stated above, SA plays a major role not only in local disease resistance (PTI and ETI) against (hemi)biotrophic pathogens, but also in **systemic acquired resistance** (**SAR**). Therefore, the next part of this introduction focuses on SAR and the most important signaling partners involved in this induced/systemic resistance response.

Once plant defense responses are activated at the site of infection, a systemic defense response is often triggered in distal plant parts to protect these undamaged tissues against subsequent invasion by pathogens. This long lasting and broad spectrum induced disease resistance is referred to as SAR and plays a central and fundamental role in protecting plants from pathogen attack (Durrant and Dong 2004). Interestingly, the SAR state can be inherited across generations (transgenerational SAR (Luna, Bruce et al. 2012)).

To establish SAR, systemic signals move from the infected to the systemic parts of the plant during a primary or 'local' infection. Subsequently, upon a secondary or systemic infection, defenses appear to be turned on faster and with a higher magnitude than in uninduced plants (Conrath 2006). Thus, SAR is characterized by an enhanced ability for a

quick and effective activation of cellular defense responses that are not induced until challenge with a pathogen. Such a state of enhanced capacity to activate stress-induced defense responses has been called the ´primed´state of the plant (Conrath 2009). Priming can also be induced by certain plant hormones or hormone-like compounds, including β-aminobutyric acid (BABA) (Jakab, Cottier et al. 2001).

SAR is characterized by the coordinate induction of a set of ***PR*** (***PATHOGENESIS-RELATED***) genes both in the infected and in the systemic tissues (Pieterse, Leon-Reyes et al. 2009), (VLOT, DEMPSEY ET AL. 2009). Many *PR* genes encode proteins with antimicrobial activity. More recently, the definition of PR proteins and other defense-related proteins has been broadened to include intra- and extracellular proteins that accumulate in intact plant tissue or cultured cells after pathogen attack or elicitor treatment (Mur, Kenton et al. 2008) (Stintzi, Heitz et al. 1993). *PR1*, for instance, is a molecular marker for SAR in the systemic tissue (Ryals, Uknes et al. 1994).

SAR is dependent upon the action of **SA** (Vlot, Dempsey et al. 2009) (Delaney, Uknes et al. 1994), which is produced locally and systemically during SAR establishment (Meuwly, Molders et al. 1995) (Raskin 1992). The necessity of SA for SAR has been confirmed in various studies. These include analyses of changes in the concentration of SA and its conjugates in infected leaves prior to, during, and after resistance. Moreover, treatment of plants with SA or various SA mimics such as acibenzolar-S-methyl (Friedrich, Lawton et al. 1996) [other names: synthetic benzo(1,2,3)thiadiazole-7-carbothioic acid(S)-methyl ester, BION, BTH, Actiguard®] and isonicotinic acid (INA) (Narusaka, Narusaka et al. 1999) (Kessmann, Staub et al. 1994) resulted in an induction of systemic defense. Finally, the link between SA and SAR was established, when transgenic plants were used in tobacco and *Arabidopsis* expressing the bacterial gene *nahG*. The *nahG* gene encodes a salicylate hydroxylase – an enzyme that is able to convert SA into catechol – and hence, the transgenic plants are unable to accumulate SA. These transgenics are not able to mount a SAR response and are more susceptible to pathogens than wild type plants (Gaffney, Friedrich et al. 1993) (Delaney, Uknes et al. 1994). This leads automatically to the question, if SA is the systemic SAR signal? Grafting experiments with chimeric grafted tobacco Xanthi-nc and *nahG* plants showed, that SA is not the translocated mobile signal responsible for inducing SAR (Vernooij, Friedrich et al. 1994). Although SA is essential for the induction of SAR (Gaffney, Friedrich et al. 1993), it is required in the systemic, SAR signal-perceiving, but not in the local, SAR signal-emitting tissue.

The elucidation of signals that are actually being transported from the site of infection to the systemic tissue remains one of the biggest tasks in SAR research for the next years (Attaran, Zeier et al. 2009) (Park, Kaimoyo et al. 2007) (Park, Liu et al. 2009). Several

different potential **signaling molecules** have been identified to date, e.g. methyl-salicylate (MeSA) (Park, Kaimoyo et al. 2007), JA (Truman, Bennett et al. 2007), azelaic acid (AzA) (Jung, Tschaplinski et al. 2009), the lipid-transfer protein DIR1 (Maldonado, Doerner et al. 2002), an SFD1/GLY1-derived glycerol-3-phosphate (G3P) (Mandal, Chanda et al. 2011) (Chanda, Xia et al. 2011), the abietane diterpenoid dehydroabietinal (DA) (Chaturvedi, Venables et al. 2012), the amino acid-derivative pipecolic acid (Pip) (Dempsey and Klessig 2012) and other lipids or lipid-derived molecules (Maldonado, Doerner et al. 2002), peptides, or proteins (Vlot, Klessig et al. 2008) (Shah 2009). Their mode of action is relatively poorly understood and multiple signals may cooperate for optimal induction of systemic defense (Vlot, Klessig et al. 2008).

Having all these signals identified as possible SAR-signals, the next step is to integrate these signals in a model, which shows the cooperative work between these signals in order to activate SAR (Dempsey and Klessig 2012). Some examples are highlighted. Mixed petiole exudates from pathogen-inoculated *dir1* and *sfd1* mutant plants induced SAR in the systemic leaves of wild type *Arabidopsis* plants. By contrast, individual *dir1* and *sfd1* petiole exudates did not induce SAR suggesting that a DIR1-dependent activity and G3P cooperate to induce SAR (Chaturvedi, Krothapalli et al. 2008). Interestingly in that respect, G3P and DIR1 appear to be mutually interdependent for translocation to systemic leaves (Chanda, Xia et al. 2011). *DIR1* is also required for AzA-induced resistance in *Arabidopsis* to infection with *Pseudomonas syringae* (Jung, Tschaplinski et al. 2009) and is needed for the induction of SAR when DA is applied locally (Chaturvedi, Venables et al. 2012). Finally, (Liu, von Dahl et al. 2011) showed that an *SFD1*-dependent SAR signal, possibly G3P together with DIR1, regulates hydrolysis of the long distance signal MeSA to its bioactive derivative SA. Together, these findings suggest a central role for *DIR1* and/or G3P in SAR. DA, in turn, also is linked to MeSA accumulation, which was shown after local DA application in wild type Arabidopsis. MeSA as well as the transcript of *AtMES9*, MeSA esterase 9, accumulated in both the treated and systemic leaves (Jaskiewicz, Conrath et al. 2011).

The work presented here focuses on proteins as potential SAR signaling factors. Other studies indicate a role for proteins or peptides in SAR signaling (Xia, Suzuki et al. 2004) (Wang, Weaver et al. 2005). The apoplastic aspartic protease CDR1, for instance, is able to cleave an unknown protein into smaller peptides, which subsequently induce a systemic defense response (Xia, Suzuki et al. 2004, Simoes, Faro et al. 2007, Lascombe, Bakan et al. 2008). Ectopic expression of rice (*Os*)CDR1 leads to constitutive activation of defense responses in rice and *Arabidopsis* (Prasad, Creissen et al. 2009) (Prasad, Creissen et al. 2010).

In addition to plant-derived peptides, pathogen-derived protein/peptide elicitors can induce SAR. Two examples are PemG1 and PeaT1. The protein elicitor PemG1 from

Magnaporthe grisea is reported, if applied purely, to induce SAR in rice and *Arabidopsis* plants (Peng, Qiu et al. 2011). Another elicitor PeaT1 from *Alternaria tenuissima* was characterized in the same way using the pathosystem tobacco and tobacco mosaic virus (TMV) (Zhang, Yang et al. 2010).

Summing up, Figure 5 shows how the translocation of mobile signaling may function, including and combining the signaling molecules involved, the potential immune reaction(s) and the reactions of the plant both in the infected, SAR signal-emitting tissue and in the uninfected, systemic tissue. In the local, infected leaf (shown in brown) three identified mobile immune SAR-signaling molecules (G3P, MeSA, AzA), which are produced after pathogen infection, are shown. These mobile immune signals are transported through the vascular tissue, leading to the accumulation of SA in the systemic, uninfected leaves. Three different SA-downstream reactions are shown leading to (1) priming of immune-related genes (via chromatin modifications), (2) transgenerational immune memory (via BRCA2) (Wang, Durrant et al. 2010) (Luna, Bruce et al. 2012) and (3) secretion of proteins with antimicrobial activity (e.g. PR proteins).

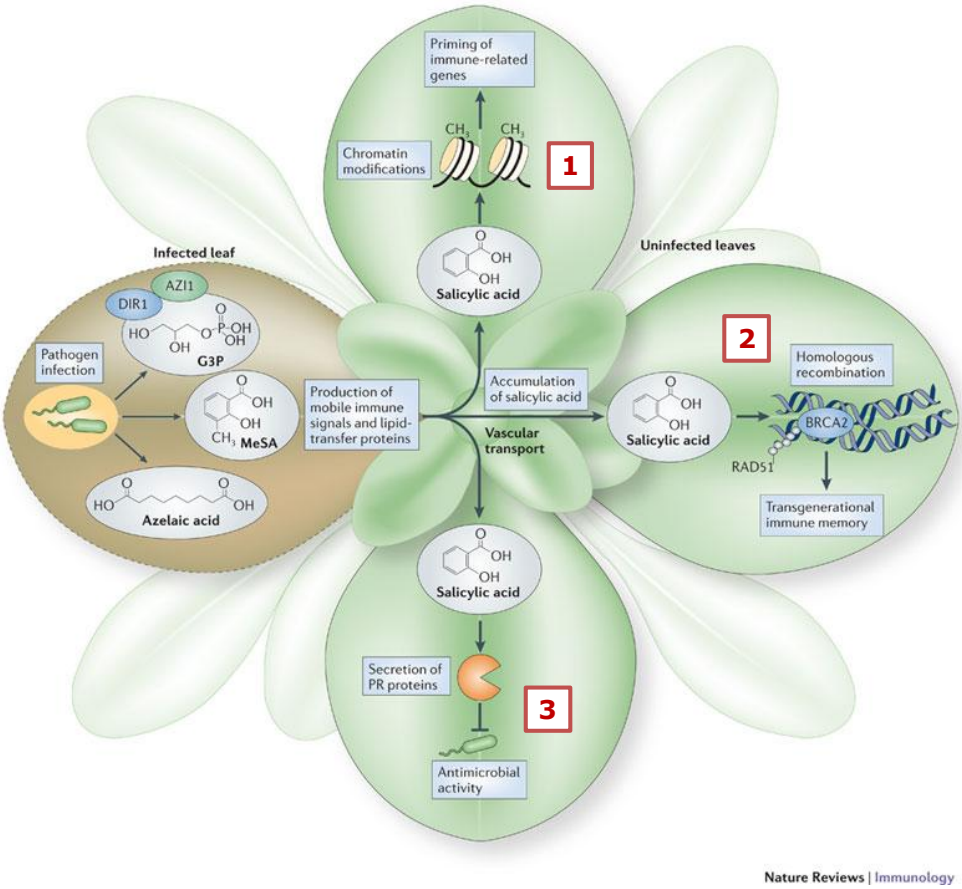


Figure 5: Translocation of mobile immune signals induces systemic immunity and immune memory.

Pathogen infection in the local tissue results in the production of mobile immune signals such as methylsalicylate (MeSA), azelaic acid and glycerol-3-phosphate (G3P), and the lipid-transfer proteins defective in induced resistance 1 (DIR1) and azelaic acid induced 1 (AZI1). These mobile signals are transported through the vasculature to systemic, uninfected parts of the plant. There they induce through an unknown mechanism

the accumulation of salicylic acid (SA). Accumulation of SA is responsible for: the secretion of pathogenesis-related (PR) proteins with antimicrobial activities, histone methylation and other chromatin modifications that prime immune-related genes for increased expression and establish immune memory, and somatic homologous recombination through the actions of BREAST CANCER SUSCEPTIBILITY 2 (BRCA2) and RAD51 to potentially establish a transgenerational memory of immunity.

Figure modified from (Spoel and Dong 2012); Copyright Agreement obtained

1.6 EDS1 - a central player in SAR

A major controller of SA signaling is encoded by the nucleo-cytoplasmic basal resistance regulator ***ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)*** (Wiermer, Feys et al. 2005) (Garcia, Blanvillain-Baufume et al. 2010). EDS1 is a lipase-like protein of unknown biochemical function. It interacts with its homologs PAD4 (phytoalexin deficient 4 – also a lipase-like protein) (Jirage, Tootle et al. 1999) and SAG101 (senescence-associated gene 101) (Feys, Wiermer et al. 2005). Together, EDS1, PAD4, and SAG101 are required for PTI as well as ETI downstream from TIR-type R proteins (Feys, Moisan et al. 2001) to signal isolate-specific pathogen recognition.

Because SA-mediated defense is reconstituted by treatment of *eds1* and *pad4* mutant plants with SA, the main function of EDS1 and its interaction partners appears to lie upstream from SA. At the same time, expression of *EDS1* and *PAD4* is enhanced by SA, indicating that *EDS1* and *PAD4* are part of an SA-dependent positive feedback loop propagating defense (Wiermer, Feys et al. 2005). Simultaneously, EDS1 and PAD4 mediate antagonism between the SA and JA/ET defense response pathways.

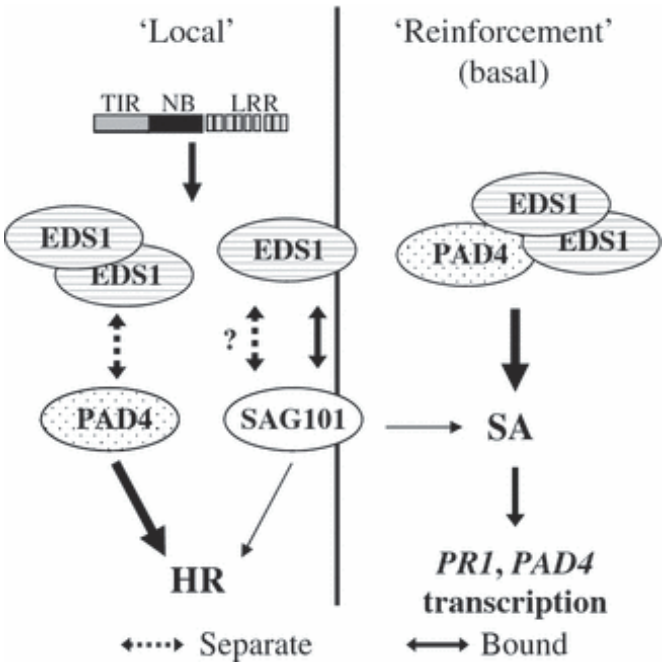


Figure 6: Model for functions of different EDS1 and PAD4 molecular configurations.

The plant immune response triggered by TIR-NB-LRR receptor recognition of an avirulent pathogen can separated into a local acute reaction and a reinforcement phase in surrounding cells leading to systemic resistance. Whereas EDS1 dissociated from PAD4 is able to confer wild-type local resistance and cell death, an EDS1-PAD4 complex is nessecary for the reinforcement/basal resistance reactions associated with transcriptional amplification of defenses through SA.

Figure taken from (Rietz, Stamm et al. 2011); Copyright Agreement obtained

During defense EDS1 is shuttled between cytoplasm, where it forms EDS1-EDS1 homodimers as well as heterodimers with PAD4, and nucleus, where it forms EDS1-SAG101 and EDS1-PAD4 heterodimers (Garcia et al., 2010) (Wiermer, Feys et al. 2005) (Parker, Holub et al. 1996). Both nuclear, and cytosolic EDS1 are required for defense. Cytosolic EDS1 is important for programmed cell death, whereas nuclear EDS1 is able to force e.g. transcriptional reprogramming essential for resistance to biotrophic and hemi-biotrophic pathogens. Most likely, the maintainance of the balance between the two EDS1 pools is important for resistance. Recent findings suggest that EDS1 connects recognition of pathogen effectors, such as AvrRps4 from *P. syringae*, to nuclear responses leading to genetic reprogramming of infected cells towards defense (Heidrich, Wirthmueller et al. 2011), (Bhattacharjee, Halane et al. 2011). The R-protein RPS4 engages EDS1 to intercept AvrRps4 and transduce receptor activation to downstream defenses.
Although ETI in response to CC-type R proteins occurs independently of EDS1 and partners, SAR downstream from the CC-type R protein RPM1 is abolished in *eds1* mutant plants (Truman, Bennett et al. 2007). Thus, in addition to PTI and ETI, EDS1 plays an important role for SAR. (Rietz, Stamm et al. 2011) showed that the interaction between EDS1 and PAD4 modulates basal resistance and SAR.

1.7 The hunt for EDS1-dependent SAR signals – preliminary work to this thesis

As mentioned above, EDS1 is required for SAR downstream from the CC-type R protein RPM1 (Truman et al., 2007). To test if EDS1 is required in the locally infected tissue for SAR signal generation or transmission or systemically for signal recognition or amplification, petiole exudates were collected from wild type and *eds1* mutant *Arabidopsis* plants after infection of the leaves with *Pseudomonas syringae* pv. *tomato* carrying the bacterial effector gene *AvrRpm1*. *AvrRpm1* activates RPM1. Petiole exudates from infected wild type plants induced *PR1* gene expression in healthy wild type plants, but not in the *eds1* mutant. This indicates that EDS1 is required for SAR signal recognition or amplification. Furthermore, petiole exudates from infected *eds1* mutant plants did not induce *PR1* gene expression in healthy wild type plants. This showed that EDS1 also is required for SAR signal generation or transmission during ETI and allows us a unique opportunity to distinguish between local ETI-related signals and systemic SAR signals in *eds1* mutant compared to wild type leaves (Jorda, Vlot, and Parker, unpublished).

By comparing the apoplastic proteomic profile of *Arabidopsis thaliana* Col-0 (able to generate a SAR signal) and *eds1* mutant plants (not able to generate a SAR signal) we are investigating which proteins possibly play a role in SAR signaling. SAR signal

generation is induced by conditional expression of the *P. syringae*-derived effector AvrRpm1 from a dexamethasone (DEX)-inducible transgene. In preliminary experiments, localized, DEX-inducible expression of *AvrRpm1*-HA induced resistance to *Pseudomonas syringae* pv. *tomato* in systemic, non-*AvrRpm1*-expressing tissues (Vlot, Wittek, and Parker, unpublished). This indicates that SAR is induced by *AvrRpm1*-triggered ETI alone and does not require additional pathogen-derived components. Hence, an initial SAR-inducing infection can be mimicked by conditional over-expression of the bacterial effector *AvrRpm1*.

In this thesis, I will apply an integrated proteomics approach to compare protein accumulation in the apoplast of AvrRpm1-expressing wild type vs *eds1* mutant plants. This experimental setup should result in the identification of proteins, which either (1) are directly transported in the plant, (2) have an enzymatic function, which generates a signal that is involved in SAR, or (3) have a signaling function resulting in SAR signal generation or transmission. By using 2D gel analysis of apoplast extracts from *AvrRpm1*-expressing wildtype and *eds1* plants gel spots were identified that accumulate in the apoplast of *Arabidopsis* in an *EDS1*-dependent manner (Vlot, Colby, and Parker conducted the analysis at the Max Planck Institute for Plant Breeding Research, Cologne, Germany - unpublished). Upon identification of these spots by mass spectrometry, seven were truly apoplastic rather than cytosolic contaminants. The seven corresponding genes were named *AtAED1-7*, which stands for ***Arabidopsis thaliana APOPLASTIC, EDS1-DEPENDENT 1-7***. *AtAED1*, encoding a predicted aspartyl protease, as well as *AtAED4* and *AtAED5*, both encoding predicted GDSL-motif lipases, are studied in this thesis. These three *At*AEDs are transiently expressed in leaf tissue of *Nicotiana tabacum* (tobacco). Subsequently, their impact systemic resistance to tobacco mosaic virus (TMV) infection is evaluated.

1.8 Integrated proteomic approach to identify potential SAR-involved proteins

Classical proteomic approaches, like **2D-gel** analysis (preliminary work outlined above) and **LC-MS/MS** (this thesis) have been used to analyze the proteomic profile of *AvrRpm1*-expressing *Arabidopsis* Col-0 and *eds1*. These methods are widely applied, also in plant-pathogen interaction studies (Bellafiore and Briggs 2007) (Kallenbach, Baldwin et al. 2009). We used 2D gels to get a comparative result of the apoplastic proteomic profile of Col-0 vs. *eds1*, whereas the LC-MS/MS (Domon and Aebersold 2006) analysis conducted in this thesis was aiming at a qualitative or at least a semi-quantitative conclusion (Uhlig, Jestoi et al. 2006). To supplement our proteomic analyses with a

quantitative approach, we applied **isotope coded protein labeling (ICPL)** (Schmidt 2005, Kellermann 2008) (Lottspeich, Schmidt et al. 2004, Paradela, Marcilla et al. 2010).

In plant biology, MS-based quantitative proteomics methods, either utilizing stable isotope labeling or label-free methods, have only recently started to find increasing application (Oeljeklaus, Meyer et al. 2009). To briefly summarize, labeling strategies rely on the incorporation of different isotopic mass tags into the peptides/proteins of the different samples. Relative quantitative information on proteins is obtained by comparing signal intensities or peak areas of differentially labeled peptide species extracted from the respective mass spectra (Oeljeklaus, Meyer et al. 2009).

In label-free methods, spectral counting of the number of peptide-associated MS/MS spectra is summed for each protein. Subsequently, differential protein expression can be determined by statistical analyses (Washburn, Wolters et al. 2001). This spectral counting, label-free method is best suited for relatively large changes in moderately abundant proteins (Mallick and Kuster 2010). Label-free spectral counting was used amongst others to reveal dynamic changes in the Arabidopsis plasma membrane during immune signaling (Elmore, Liu et al. 2012).

There are two stable isotope labeling options for quantitative proteomics: metabolic and chemical labeling. Metabolic labeling exploits the in vivo incorporation of stable isotopes during protein biosynthesis and is more likely to be achieved by growing cells or entire organisms. Metabolic isotope labeling can be conducted in cell culture of *A. thaliana* (SILAC - stable isotope labeling by amino acids in cell culture) (Ong, Blagoev et al. 2002) (Engelsberger, Erban et al. 2006) (Gruhler, Schulze et al. 2005) and entire *A. thaliana* plants (HILEP – hydroponic isotope labeling of entire plants) (Bindschedler, Palmblad et al. 2008).

Compared to metabolic labeling, a considerable benefit of chemical labeling emerged in the last years. Chemical labeling can be performed in vitro due to the various functional groups in proteins. The labeling reagents tag proteins or the respective proteolytic peptides. By comparing the label upon MS/MS fragmentation a quantitative statement can be given. Examples for chemical labeling are the isotope coded affinity tag (ICAT) technique (Gygi, Rist et al. 1999) (Dunkley, Dupree et al. 2004), isobaric tag for relative and absolute quantification (iTRAQ) (Ross, Huang et al. 2004), and isotope-coded protein labeling (ICPL) (Schmidt 2005) (Kellermann 2008). iTRAQ has been applied to determine changes in the phosphoproteome during the defense response of *A. thaliana* against *Pseudomonas syringae* pv. *tomato* DC3000 (Jones, Bennett et al. 2006) and to investigate potential mechanisms of disease resistance and susceptibility in the

secretome in the *Arabidopsis-Pseudomonas syringae* pathosystem (Kaffarnik, Jones et al. 2009). Figure 7 sums up the different techniques available to date in a quantitative proteomic set-up. In this thesis, ICPL was used.

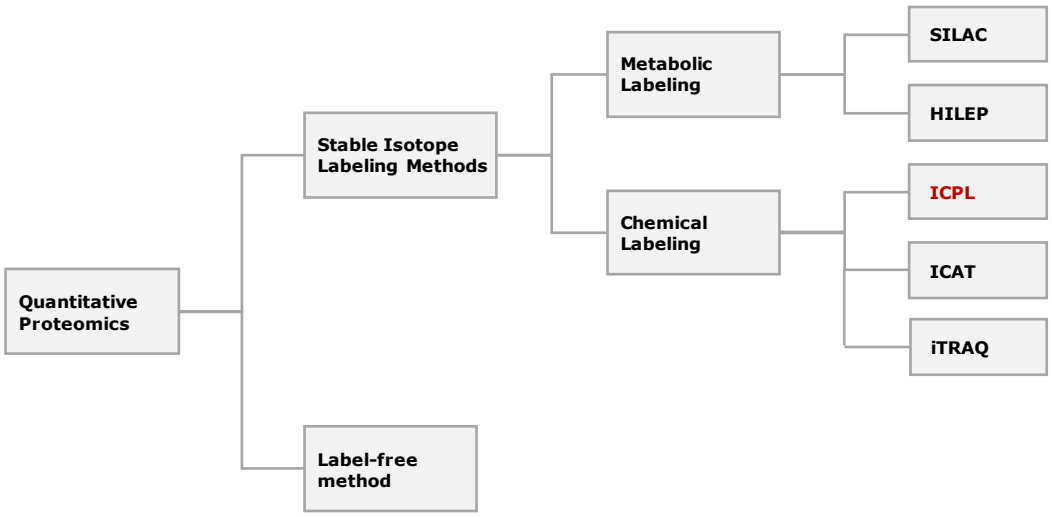


Figure 7: Quantitative proteomic methods available to date.

Abbreviation highlighted in red: ICPL, Isotope coded protein labeling; in black: SILAC, stable isotope labeling by amino acids in cell culture; HILEP, hydroponic isotope labeling of entire plants; ICAT, isotope coded affinity tag; iTRAQ, isobaric tag for relative and absolute quantification

1.9 Goals of the PhD work and strategy

The research work of this PhD project with the title "Identification of new regulators for systemic acquired resistance (SAR) in plants by an integrated proteomics approach" aimed to identify new SAR signaling partners by using a proteomics-based approach. To this end, the peptide/protein composition of the intercellular washing fluid of *AvrRpm1*-expressing Col-0 wild type and *eds1* mutant *Arabidopsis* plants was investigated and compared by LC-MS/MS analysis (liquid chromatography coupled with tandem mass spectrometry) as well as by ICPL (Isotope Coded Protein Labeling).

A role in SAR/disease resistance of individual proteins-of-interest was clarified in *Arabidopsis* plants, in which expression of the corresponding gene(s) was knocked out. The Knock-out (KO) mutants were characterized in a classical SAR experiment, in growth curve analysis with different *Pseudomonas syringae* strains as well as in gene expression experiments before and after pathogen infection. Furthermore, a potential function of SAR/resistance-inducing genes in other plant species was investigated (tobacco) (Zwicker, Mast et al. 2007).

Chapter 2: MATERIAL AND METHODS

2.1 MATERIAL

2.1.1 Plant material

Arabidopsis thaliana ecotype Col-0 was used throughout this study. The *eds1-2* mutant allele in Col-0 and the *DEX::AvrRpm1-HA* transgenic line were previously described (Bartsch, Gobbato et al. 2006) (Mackey, Holt et al. 2002). These two lines were crossed to yield *eds1-2 DEX::AvrRpm1-HA* (Vlot and Parker, unpublished). T-DNA Insertion mutant lines *aed9-1* and *aed9-3* (Table 1) were identified by screening the SIGnAL T-DNA Express database of the SALK Institute (http://signal.salk.edu/cgi-bin/tdnaexpress). The mutants were retrieved from the Nottingham Arabidopsis Stock Center (NASC) (Alonso, Stepanova et al. 2003).

Table 1: T-DNA-insertion mutants.

Locus	SALK Line	Name	Ecotype	Insertion sequence
*At*3G15356	SALK_030762	*aed9-1*	Col-0	pBIN-pROK2
*At*5G03350	SALK_036814	*aed9-3*	Col-0	pBIN-pROK2

Nicotiana tabacum (tobacco) Xanthi nc containing the TMV resistance gene *N* (Park, Kaimoyo et al. 2007) was used alongside a Xanthi nc *NahG* transgenic line (Gaffney, Friedrich et al. 1993).

2.1.2 Soil

For *Arabidopsis* breeding, soil (Floraton 1, Floragard, Oldenburg, Germany) was mixed with silica sand in a ratio of 5:1 and poured in 4-well plant pots. Soil was wetted with water, seeds where placed with a toothpick on wet soil and stratified for 2 days at 4°C (to synchronize germination) before transfer into the plant chamber.
For *Nicothiana tabacum* Xanthi nc and Xanthi nc *NahG* breeding, soil (Einheitserde Classic, Einheitserde- und Humuswerke, Gebr. Patzer GmbH, Sinntal-Jossen, Germany) was mixed with perlite (Knauf, Iphofen, Germany) in a ratio of 5:1 and poured in 4-well plant pots. Soil was wetted with water, seeds where placed with a toothpick on wet soil

and transferred to the plant chamber. After two weeks, plants were individualized and transferred to a bigger pot.

2.1.3 Bacterial strains

Bacteria used in this study are summarized in Table 2. They are sorted by the species as well as by the different strains used.

Table 2: Bacterial strains.

Species	Strain
Escherichia coli	DH-5α
Pseudomonas syringae	*pv tomato* (DC3000) – *AvrRpm1* - avirulent
	pv tomato (DC3000) – *AvrRps4* - avirulent
	pv tomato (DC3000) – virulent
Agrobacterium tumefaciens	GV3101

2.1.4 *Tobacco mosaic virus* (TMV) preparation

Table 3 shows the different buffers and chemicals used for preparation and isolation of TMV.

Table 3: Buffers and chemicals used for the preparation/isolation of TMV.

Name	Composition	Source
Phosphatebuffer, pH 7	0.5M Na_2HPO_4	Merck, Darmstadt, Germany
Virion extraction buffer	1% 2-mercaptoethanol was added to 0.5M phosphatebuffer	Sigma, Taufkirchen, Germany
Sea sand		Merck, Darmstadt, Germany
Butanol		Merck, Darmstadt, Germany
Polyethylene glycol (PEG)	20% PEG solution	Roth, Karlsruhe, Germany
NaCl solution	5M NaCl	Roth, Karlsruhe, Germany

2.1.5 Vectors

Table 4 summarizes vectors used for various cloning purposes. pENTR™/D-TOPO® was used as the entry vector for all cloning strategies. pER8-GW-Cterm-HAStrep was derived from pER8 (Zuo, Niu et al. 2000) and obtained from Jane Parker, Max Planck Institute for Plant Breeding Research. It was used as expression/destination vector to support expression of genes-of-interest in plants. pER8-GW-Cterm-HA-Strep links a 3XHA-strep tag to the C-terminal end of the encoded proteins (Bautor and Parker, unpublished. Transgene expression *in planta* is driven by the estradiol-inducible XVE promoter (Zuo, Niu et al. 2000).

pHANNIBAL was used in the cloning strategy for the silencing constructs (Wesley, Helliwell et al. 2001). All constructs were subcloned into the binary vector pART27 (Wesley, Helliwell et al. 2001).
pHANNIBAL and pART27 were obtained from Dan Klessig´s Lab, Boyce Thompson Institute, Cornell University, Ithaca, New York, USA. Table 4 summarizes the vectors used in the present study, classified according to their application and their source of supply.

Table 4: Vectors.

Name of vector	Application	Source
pENTR™/D-TOPO® vector	Gateway™ cloning (entry vector)	Invitrogen, Karlsruhe, Germany
pER8-GW-Cterm-3XHA Strep	Gateway™ cloning (destination/expression vector)	Bautor and Parker, Max Planck Institute for Plant Breeding Research, Cologne, Germany
pHANNIBAL	RNAi silencing	Dan Klessig´s Lab; Boyce Thompson Institute, Ithaca, New York, USA
pART27	RNAi silencing (plant expression vector)	Dan Klessig´s Lab; Boyce Thompson Institute, Ithaca, New York, USA

2.1.6 Chemicals

All chemicals used in this doctoral thesis were obtained in high purity grade and purchased either from Sigma Aldrich GmbH (Taufkirchen, Germany, consecutively named Sigma), Carl Roth GmbH (Karlsruhe, Germany) or from other sources as indicated directly in the tables.

2.1.7 DNA-Polymerases

Table 5 summarizes the DNA-polymerases applied. Proof-reading polymerases were used in cloning procedures; the Mango *Taq*™ DNA-polymerase was mainly employed in colony-PCRs and SYBR green polymerases were used in qPCR analysis.

Table 5: Different DNA-polymerases used in presented work.

Name	Source	Specifications
Phusion® High Fidelity DNA Polymerase	Finnzymes, Vantaa, Finland	Proof-reading polymerase
iProof High Fidelity DNA Polymerase	Bio-Rad, München, Germany	Proof-reading polymerase
Mango *Taq*™ DNA Polymerase	Bioline, Luckenwalde, Germany	
Absolute QPCR SYBR Green Low ROX Mix	Thermo Scientific, Bonn, Germany	Polymerase used in qPCR analysis
SensiMix™ SYBR Low-ROX Kit	Bioline, Luckenwalde, Germany	Polymerase used in qPCR analysis

2.1.8 Enzymes used for restriction, cDNA-synthesis and Gateway® cloning

The FastDigest Enzymes were used in restriction application during the cloning procedure of the RNAi constructs. T4 Ligase was applied in order to re-ligate digested fragments. FastAP™ Thermosensitive Alkaline Phosphatase dephosphorylizes DNA to prevent re-circularization.

Table 6: Enzymes used for restriction, cDNA-synthesis and Gateway® cloning

Name	Used in	Source
FastDigest® *BamHI, HindIII, KpnI, NotI, XhoI*	RNAi silencing cloning procedure	Fermentas, St. Leon-Rot, Germany
FastAP™ Thermosensitive Alkaline Phosphatase	RNAi silencing cloning procedure	Fermentas, St. Leon-Rot, Germany
T4 DNA Ligase	RNAi silencing cloning procedure	Fermentas, St. Leon-Rot, Germany
SuperScript™II Transcriptase	cDNA-synthesis	Invitrogen, Karlsruhe, Germany
SuperScript™III Transcriptase	cDNA-synthesis	Invitrogen, Karlsruhe, Germany
RNaseOut™	cDNA-synthesis	Invitrogen, Karlsruhe, Germany
Gateway® BP Clonase™ Enzyme Mix	Gateway® cloning	Invitrogen, Karlsruhe, Germany
Gateway® LR Clonase™ Enzyme Mix	Gateway® cloning	Invitrogen, Karlsruhe, Germany

2.1.9 Primers

Primers from the following tables were applied in various cloning strategies. The working concentration of the primers was 5µM for PCR and 10µM for qPCR. The fragment sizes of the resulting products as well as the annealing temperatures of the primers are specified for every primer pair. Tables of primers presented in detail in the supplemental part of this thesis.

2.1.10 Antibiotics

Table 7 shows the different antibiotics used in various applications with their concentration in a stock solution as well as with their final working concentration.

Table 7: Antibiotic stock solution and working concentrations.

	Stock solution (mg/ml)	Working concentration (µg/ml)	Source
Ampicillin	50	50	Roth, Karlsruhe, Germany
Gentamycin	25	25	Roche, Mannheim, Germany
Kanamycin	50	50	Roth, Karlsruhe, Germany

Table 7 continued	**Stock solution**	**Working concentration**	**Source**
Rifampycin	100	100	Duchefa Biochemie, Germany
Spectinomycin	100	100	Sigma, Taufkirchen, Germany
Carbenicilin	100	250	Roth, Karlsruhe, Germany
Cefotaxim	100	100	AppliChem, Darmstadt, Germany
Hygromycin	50	50	Roth, Karlsruhe, Germany

All stock solutions were dissolved in water except Rifampycin. Rifampycin was dissolved in 100% Dimethylsulfoxide (DMSO). Stock solutions were filter sterilized through 0.22 µm sterile filters (Millipore, Billerica, MA, United States) and stored as aliquots at -20°C. The aqueous hygromycin stock solution was purchased ´ready-to-use´ (Roth, Karlsruhe, Germany).

2.1.11 Western Blot reagents

Table 8: Antibodies and luminescence reagent used in western blot analysis.

	Product Information	**Source**
Primary antibody	Anti-HA antibody produced in rabbit	Sigma, Taufkirchen, Germany
Secondary antibody	Anti-Rabbit lgG (H+L), HRP conjugate	Promega, Mannheim, Germany
Luminescence reagent	Immun-Star™ WesternC™ Kit	Bio-Rad, München, Germany

2.1.12 Media, solutions and buffers

Table 9 illustrates the buffer compositions and the required chemicals for the phenol-chloroform-based RNA extraction (Logemann, Schell et al. 1987).

Table 9: Buffers and chemicals used for RNA extraction.

Name	Composition	Source
TriReagent, per 100ml (adjust pH 5.0 before adding Phenol)	3.05 g Ammoniumrhodanide (Ammoniumthiocyanate)	Roth, Karlsruhe, Germany
	9.44 g Guanidinthiocyanat	Merck, Darmstadt, Germany
	5 ml Glycerol	Roth, Karlsruhe, Germany
	3 M Na-Acetate pH 5.2	Merck, Darmstadt, Germany
	40 ml H_2O	
	38 ml Phenol	Roth, Karlsruhe, Germany
Solutions used	Chloroform	Merck, Darmstadt, Germany
	2-Propanol	Roth, Karlsruhe, Germany
	Ethanol	Merck, Darmstadt, Germany

Table 10: Media used for bacteria cultivation.

Name	Composition	Source
NYGA, per liter, pH 7.0	5g Bacto-proteose Peptone	Roth, Karlsruhe, Germany
	3g yeast extract	Roth, Karlsruhe, Germany
	20ml Glycerol	Roth, Karlsruhe, Germany
	18g Agar-Agar	Merck, Darmstadt, Germany
Culture media, Luria broth medium (LB), per liter, pH 7.4	10g Tryptone	Roth, Karlsruhe, Germany
	5g yeast extract	Roth, Karlsruhe, Germany
	10g NaCl	Roth, Karlsruhe, Germany

Table 11: Apoplast extraction buffers.

Name/Application	Composition (per liter)	Source
APO-Buffer I → Used for subsequent LC-MS/MS analysis	2.5mM Tris; pH 7.4	Roth, Karlsruhe, Germany
	1mM EDTA	Sigma, Taufkirchen, Germany
	30mM $MgCl_2$	Roth, Karlsruhe, Germany
APO-Buffer II → Used for subsequent ICPL analysis	2.5mM HEPES; pH 7.4	Sigma, Taufkirchen, Germany
	1mM EDTA	Sigma, Taufkirchen, Germany
	30mM $MgCl_2$	Roth, Karlsruhe, Germany

Table 12: Buffers and solutions used in Western blot analysis.

Name	Composition	Source
Running Gel Solution, 10%	Water	
	1.5M Tris HCL, pH 8.8	Roth, Karlsruhe, Germany
	20% SDS	Merck, Darmstadt, Germany
	Acrylamid	AppliChem, Darmstadt, Germany
	10% APS	Roth, Karlsruhe, Germany
	TEMED	AppliChem, Darmstadt, Germany
Stacking Gel Solution, 4%	Same composition used as in Running Gel Solution, change:	
	0.5M Tris-HCL, pH 6.8	Roth, Karlsruhe, Germany
2X SDS protein gel loading buffer	0.125M Tris-HCL, pH 6.8	Merck, Darmstadt, Germany
	4% SDS	Merck, Darmstadt, Germany
	20% glycerol	Roth, Karlsruhe, Germany
	0.3M ß-mercaptoethanol	Sigma, Taufkirchen, Germany
	0.05% bromophenol blue	Merck, Darmstadt, Germany
5x Electrophoresis Buffer,	0.125M Tris-base	Merck, Darmstadt, Germany

pH 8.3	0.96M glycine	Sigma, Taufkirchen, Germany
	0.5% SDS	Merck, Darmstadt, Germany
Electrotransfer buffer, per liter	25mM Tris-base	Merck, Darmstadt, Germany
	192mM glycine	Sigma, Taufkirchen, Germany
	20% methanol	Roth, Karlsruhe, Germany
	0.1% SDS	Merck, Darmstadt, Germany
PBS, pH 7.4	10mM NaH_2PO_4	Merck, Darmstadt, Germany
	120mM NaCl	Merck, Darmstadt, Germany
	2.7mM KCl	Merck, Darmstadt, Germany
PBS-Tween	PBS see above	
	0.05% Tween-20	Sigma, Taufkirchen, Germany

Table 13: Media used to grow plants in a sterile environment.

Name/Application	Composition (per liter)	Source
Sterile plant growth medium	4.302g Murashige & Skoog	Duchefa Biochemie, Germany
	10g Sucrose	Duchefa Biochemie, Germany
	0.5g MES	Roth, Karlsruhe, Germany
	After adjusting pH 5.0, 8g of Agar/Gelrite were added	Roth, Karlsruhe, Germany

After autoclaving, different compounds for the selection process were added to the media, which was cooled down to around 55°C. Carbenicilin, Hygromycin and Cefotaxim were added to select the plants over-expressing a gene-of-interest. For the RNAi-selection-medium Carbenicilin, Kanamycin and Cefotaxim were added. Agar instead of gelrite was used in experiments, in which the selection was conducted based on kanamycin.

Table 14: Buffers used in a SAR experiment.

Name	Composition	Source
MOCK solution and dilution buffer, pH 7.0	10mM $MgCl_2$	Merck, Darmstadt, Germany
Bacteria isolation buffer	10mM $MgCl_2$	Merck, Darmstadt, Germany
	0.01% Silwet	Lehle Seeds, Texas, USA

MOCK solution and dilution buffer was used to either infiltrate the control plants (MOCK-treatment) or to dilute the *Pseudomonas* strains to a certain concentration in order to infiltrate *Arabidopsis* leaves. The application of the bacteria isolation buffer was to isolate bacteria out of infected leaves/leaf discs with subsequent determination of the bacterial content by using a dilution series.

Table 15: Buffer and media used for *Agrobacterium* infiltration.

Name	Composition	Source
Min A, per liter	10.5g K_2HPO_4	Merck, Darmstadt
	4.5g KH_2PO_4	Merck, Darmstadt, Germany
	1.0g Ammonium sulphate	Merck, Darmstadt, Germany
	0.5g sodium citrate x H_2O	Sigma, Deisenhofen, Germany
	1ml 1M $MgSO_4$	Sigma, Deisenhofen, Germany
	10ml 20% glucose	Roth, Karlsruhe, Germany
	0.1ml 1M $CaCl_2$	Merck, Darmstadt, Germany
MES, pH 5.6	100mM MES	Boehringer Ingelheim, Ingelheim, Germany
MMA	10mM MES	Boehringer Ingelheim, Ingelheim, Germany
	10mM $MgCl_2$	Merck, Darmstadt, Germany
	200µM Acetosyringone	Sigma, Deisenhofen, Germany

Table 16: Buffer used for TMV infection of tobacco plants.

Name	Composition	Source
Phosphatebuffer, pH 7.0	0.5M NaH_2PO_4	Merck, Darmstadt, Germany
	0.5M KH_2PO_4	Merck, Darmstadt, Germany

2.1.13 Kits

Table 17: Kits and filter units used in presented work.

Kit	Source	Application
SERVA ICPL™ Kit	Serva Electrophoresis, Heidelberg, Germany	ICPL-analysis of apoplast extracts
QIAprep® Spin Miniprep Kit, No. 27104	Qiagen GmbH, Hilden, Germany	Isolation of plasmid DNA from bacteria
QIAquick® Gel Extraction Kit, No. 28704	Qiagen GmbH, Hilden, Germany	Extraction of DNA from an agarose-gel
QIAquick® PCR Purification Kit, No. 28104	Qiagen GmbH, Hilden, Germany	Purification of a PCR product
Filter	**Source**	**Application**
Amicon Ultra – 0.5ml 3K; Ultracel® - 3K Membrane	Millipore, Schwalbach, Germany	Pre-cleaning and size-exclusion of apoplast extracts
Bond Elut – C18, 100MG, 1ML	Agilent Technologies, Oberhaching, Germany	Cleaning of apoplast extracts prior to LC-MS/MS analysis

2.2 METHODS

2.2.1 Plant growth conditions

Arabidopsis were grown in 10 hour light – 14 hour dark cycles at 70% relative humidity and 22°C. The light intensity during the light period was kept at 100 µE m^{-2} sec^{-1}.

Nicotiana tabacum cv. Xanthi *nc* and *Nicotiana tabacum* Xanthi *nc nahG* were grown in cycles of 14 hours of light (approx. 200 µE m^{-2} s^{-1}) at 25°C and 10 hours of darkness at 20°C. The relative humidity was set constantly to 70%.

Controlled climate conditions were assured by cultivation of all plants in growth chambers.

2.2.2 Seed storage and surface sterilization

Seeds were harvested and stored in a paper bag, which was permeable to air, in a dark place at room temperature.
For surface sterilization, *A. thaliana* seeds were first rotated in 70% Ethanol for one minute. Afterwards the ethanol was decanted and the seeds were incubated in 10% DanKlorix (Colgate-Palmolive, Hamburg, Germany) solution for 15 minutes. Subsequently, the DanKlorix solution was decanted and the seeds were washed with autoclaved ddH_2O for four times.

2.2.3 Bacterial Strains and Culture Conditions

Pseudomonas syringae pathovar *tomato* (referred to as DC3000 or *Pst*) carrying *AvrRpm1* or *AvrRps4* was maintained on NYGA medium. Selective antibiotics were rifampicin and kanamycin. The bacteria were grown at 28 °C in the incubator (MMM-Friocell 111, Munich, Germany) 2X O/N.
Escherichia coli DH5α (Invitrogen™) was cultured in LB medium at 37 °C in the incubator (Memmert 500, Nuremberg, Germany) O/N.
Agrobacterium tumefaciens strain GV3101 was cultured in LB medium at 28 °C. Antibiotics used were rifampicin, gentamicin and spectinomycin (for selection of plasmids).

Competent *E. coli* cells for routine cloning were gained by the RbCl transformation protocol of New England Biolabs (NEB, Norwich, England).

2.2.4 *Tobacco mosaic virus* (TMV) isolation

TMV isolation was done according to (Chapman 1998) and the buffers needed are described in Table 3 of this thesis. The TMV solution was used for the infection of *Nicotiana tabacum*. After 5 days the lesion size was measured and the lesion number counted to assign the right dilution of the TMV stock for further infections.

2.2.5 Phenol-RNA Extraction, DNA extraction and cDNA-synthese

Frozen plant material was ground and RNA was isolated by phenol-extraction method (Logemann, Schell et al. 1987). Quality and concentration of the RNA samples were determined measuring the absorption at 260 nm and 280 nm using the Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). The A260/A280 ratio was used to assess the purity of total DNA or RNA and to detect the presence of protein, phenolics or other contaminants that absorb at or near 280nm. A ratio of approximately 1.8 or 2.0 is generally accepted for pure DNA and RNA, respectively. The A260/230 ratio is a second purity measure, which should commonly be in the range of 2.0-2.2. An appreciably lower ratio may indicate the presence of contaminants absorbing at 230 nm. RNA integrity was analyzed by using 1% agarose gel electrophoresis.

Frozen plant material was ground and DNA was isolated by adding 500µl choloform and 500µl CTAB+1% β-mercaptoethanol to the samples. Everything was shaken at 1400rpm at 8°C for 15 minutes. Subsequently, the samples were centrifuged at 14000rpm at 4°C for 10 minutes. The supernatant (approx. 600µl) was added to a new reaction tube, which was supplemented with 100µl phytopure (GE Healthcare, Buckinghamshire, UK) and 500µl chlorophorm. Everything was shaken at 1400rpm at 8°C for 15 minutes. Subsequently, the samples were centrifuged at 14000rpm at 4°C for 10 minutes. The supernatant (approx. 500µl) was added to new reaction tube, which was supplemented with 250µl isopropanol. After gentle mixing the samples were incubated on ice for 10 minutes. Subsequently, the samples were centrifuged at 14000rpm at 4°C for 10 minutes. Supernatant was decanted and 1ml of 70%ethanol/0.1M sodium acetate was added to the pellet. Additionally, the samples were incubated for 5 minutes at room temperature. The pellet was washed twice, once with 80% ethanol and once with 100% ethanol, and subsequently dried. In order to resolve the pellet, 30µl purified water

(Licrosolv, Merck, Darmstadt Germany) was added. The samples were then shaken at 800rpm at 8°C for 20 minutes and stored at -20°C.

cDNA was synthesized by using SuperScript™II or SuperScript™III Reverse Transcriptase following the manufacturer's instructions (Invitrogen, California, USA).

2.2.6 PCR (Polymerase Chain Reaction) and qRT-PCR (quantitative real-time-PCR) analysis

Polymerase chain reaction (PCR) is a method that allows exponential amplification of short DNA sequences within a longer double stranded DNA molecule *in vitro*. Amplification of genes-of-interest was performed by a standard PCR protocol including the steps: denaturation, annealing of gene-specific primers, elongation. The denaturation temperature was different in different PCR reactions depending on the DNA polymerase used (Table 5). It was normally between 94°C and 98°C. The annealing temperature depended on the length and base pair composition of the primers used for amplification (Table 25, Table 26, Table 27) and is one of the most important parameters that needs adjustment in the PCR reaction. Elongation was carried out in between 70°C and 72°C depending on the different DNA-Polymerases used (Table 5). Moreover, the duration of elongation depended on the expected length of the PCR product. PCR was executed in a MJ Research PTC-200 Peltier Thermal Cycler.

Real-time PCR (qPCR) is a form of PCR in which data are collected in real-time as the reaction proceeds. Continuous data collection enables the most important application of real-time PCR, target quantification. cDNA was used as a template, in order to quantify the expression of genes-of-interest. To conduct the qRT-PCR, the 7500 Real Time PCR System from Applied Biosystems (Darmstadt, Germany) and SYBR Green mixes (Table 5) were used.

2.2.7 Nucleic acid agarose gel electrophoresis

The separation of nucleic acids according to fragment size was done in 1% to 1,5% agarose (Biozym, Hessisch Oldendorf, Germany) gels (depending on the fragment size) in 1X TAE buffer (Tris-Acetate-EDTA, final concentration 40mM Tris acetate and 1mM EDTA, both purchased from Roth, Karlsruhe, Germany). After dissolving agarose in TAE buffer by boiling and cooling down to about 50°C, 0.05 µg/ml ethidium bromide (Roth, Karlsruhe, Germany) was added and the gel was casted. Samples were mixed with 6X loading dye (Fermentas, St Leon-Rot, Germany) to a final dye concentration of 1X and

the gels were run at a voltage between 70 of 100 V, depending on the size of the gel and the size of the fragment.
After separation, nucleic acids were visualized with UV light (302 nm). Subsequently, the gels were photographed and documented using the BIO-Print M1 gel documentation system from Vilber Lourmat (Eberhardzell, Germany).

2.2.8 Purification of PCR products and DNA reaction mixtures

If necessary for downstream experiments, DNA products from PCR or after enzymatic cleavage were cleaned with the QIAquick PCR purification Kit (Table 17), according to the manufacturer´s instructions. The concentration of the DNA was analyzed by measuring the absorption at 260 nm and the purity by measuring the absorption at 280 nm and 230 nm, calculating the ratios of A_{260nm}/A_{230nm} and A_{260nm}/A_{280nm} with the Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA).
After agarose gel electrophoresis DNA bands were cut under UV light with a scalpel and transferred to a 2 ml reaction tube. DNA was extracted using the QIAquick Gel Extraction Kit (Table 17) according to the manufacturer´s instructions. The concentration and purity of the DNA was analyzed with the Nanodrop ND-1000 spectrophotometer as described above.

2.2.9 Gateway® cloning

Unless otherwise indicated the Gateway® cloning reactions were performed according to the manufacturer´s instructions (Table 6).

2.2.10 Transformation of competent *E. coli* and *A. tumefaciens* GV3101

The two methods to incorporate plasmids into bacteria used in this PhD thesis were chemical transformation as well as transformation by electroporation.

Chemical Transformation

For transformation of competent bacteria by heat shock an aliquot of 50 µl competent *E. coli* cells was thawed on ice for 10 minutes and then mixed with 1 – 20 ng plasmid DNA or 0.5 – 2 µl of an enzymatic reaction mixture. The mixture was incubated on ice for 30 minutes. Afterwards the cells were transformed by incubation at 42°C for 30 seconds with subsequent immediate cooling on ice. The cells were suspended in 1 ml LB media and shaken (250 rpm) for one hour at 37°C. Then 20 – 200 µl were plated on LB plates

with appropriate antibiotics. Single colonies were used for further experiments after incubation of the plates at 37°C O/N.

Transformation by Electroporation

For electroporation, an aliquot of 50 µl electrocompetent *E. coli* or *A. tumefaciens* cells was thawed on ice for 10 minutes, mixed with either 100 ng plasmid DNA or with 0.5 - 1 µl of an enzymatic reaction and transferred to a prechilled 1mm Gene Pulser cuvette (Bio-Rad, Munich, Germany). An electric pulse of 25 µF capacitance, 1.25 V and 400 Ω resistance was applied. Afterwards, cells were immediately suspended in 2 ml LB medium and shaken (250 rpm) for one hour at 37°C or 28°C, respectively. Then 20 – 200 µl were plated on LB plates with appropriate antibiotics. Single colonies were used for further experiments after incubation of the plates at 37°C or 28°C, respectively O/N for *E. coli* and 2X O/N for *A. tumefaciens*.

2.2.11 Arabidopsis transgenic plant production/identification including the cloning procedure of appropriate constructs and subsequent selection process of the plants

2.2.11.1 Identification of homozygous knock out plant lines

For identification of homozygous *aed9-1* and *aed9-3* (Table 1) KO mutants a PCR-based method was applied. Figure 8 illustrates the strategy and the theoretical outcome of the PCR-based method used.

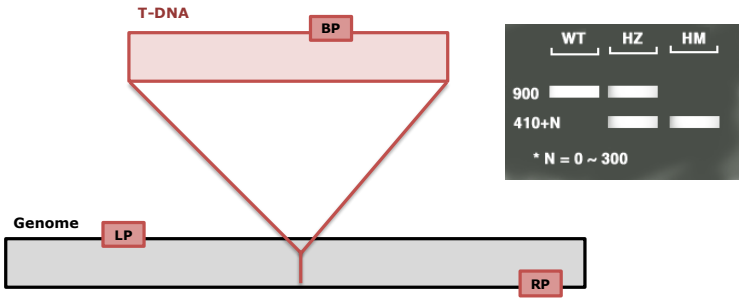


Figure 8: Illustration of PCR-based strategy to identify homozygous KO mutants.

Figure was modified from http://signal.salk.edu/tdnaprimers.2.html, WT, wild-type, HZ, heterozygous, HM, homozygous, N, difference of the actual insertion site and the flanking sequence position, usually 0 - 300 bases, LP and RP, left and right genomic primer, BP, T-DNA border primer

Primers used for screening the *aed9-1*mutants were HB-P1 (BP), HB-P2 (LP) and HB-P3 (RP) (Table 25), whereas HB-P1 (BP), HB-P4 (LP) and HB-P5 (RP) (Table 25) were taken for *aed9-3* screening. For *aed9-1* two PCR reactions were performed for each plant – one

with the primer pair HB-P2 and HB-P3 and the second reaction with HB-P1 and HB-P3. The PCR products were separated via electrophoresis on a 1% agarose gel and the occurring bands were evaluated. Plants only showing a PCR product using the primer pair HB-P1 and HB-P3 were homozygous KO mutant plants and used for further analysis. If there was only a PCR product detectable using primer pair HB-P2 and HB-P3, the plant was considered as wild-type and if both primer pairs resulted in a PCR product, the plant was heterozygous. The same procedure as described above was applied for screening *aed9-3* KO mutant plants.

2.2.11.2 Cloning of over-expression constructs

Six different over-expression constructs (*At*AED1 neighbor, *At*AED4, *At*AED5, *At*AED9-1, *At*AED9-2, *At*AED9-3) were cloned in this PhD work. They were either used in transient over-expression experiments in tobacco and/or in floral dipping (Clough and Bent 1998) of *Arabidopsis*. The construct for expression of *At*AED1 in plants was ´ready-to-use´ (Vlot et al., unpublished).

For all constructs RNA from wild type, untreated *Arabidopsis* was taken as template for a reverse transcriptase reaction with enzyme SuperScript™II or SuperScript™III Reverse Transcriptase (Table 6) using oligo-dT (5´TTT TTT TTT TTT TTT TTT TTT TTT TT 3´) as a primer. 1 µl of the resulting cDNA was used as a template for all PCR reactions described in this section. For PCR either Phusion® High Fidelity DNA Polymerase or iProof High Fidelity DNA Polymerase was used and the Gateway® cloning technique was applied (Table 5, Table 6).

For *At*AED1neighbor primer pair HB-P5 and HB-P6, for *At*AED4 primer pair HB-P7 and HB-P8, for *At*AED5 primer pair HB-P9 and HB-P10, for *At*AED9-1 primer pair HB-P11 and HB-P12, for *At*AED9-2 primer pair HB-P13 and HB-P14 and for *At*AED9-3 primer pair HB-P15 and HB-P16 were used and PCR was conducted with specific annealing temperatures for each gene as shown in Table 26.
PCR products were applied to gel electrophoresis and the band showing the correct fragment size (Table 26) was cut out. Subsequently, the PCR product was extracted using the QIAquick® Gel Extraction Kit (Table 17) as described above. The PCR products were then cloned in pENTR™/D-TOPO® according to the manufacturer´s instructions (BP-reaction) followed by the transformation of the cloning preparation into *E. coli* (DH5α). The bacteria were spread on LB plates containing the selective antibiotic kanamycin (Table 7). After incubation at 37°C O/N, resulting colonies were subjected to colony PCR using the same primers as for the respective initial PCRs. Positive colonies were transferred to liquid LB cultures containing kanamycin. After incubation at 37°C O/N, the

plasmids were isolated from the bacteria using the QIAprep® Spin Miniprep Kit according to the manufacturer´s instructions (Table 17). All cloned fragments/'inserts' were sequenced using M13 forward (TGT AAA ACG ACG GCC AGT) and M13 reverse (CAG GAA ACA GCT ATG ACC) primers (Eurofins MWG GmbH, Ebersberg, Germany). Sequences were then aligned with the respective cDNA sequences (BioEdit – Biological sequence alignment editor) from the Arabidopsis database, and plasmids containing inserts with a correct target gene sequence were used for further steps.

The target gene sequences were transferred from the respective pENTR™/D-TOPO® clones into the Gateway destination vector pER8-GW-Cterm-3XHAStrep (LR reaction, Table 4). Subsequently, thus generated pER8-GW-Cterm-3XHAStrep expression vectors were transformed into *A. tumefaciens* GV3101 via electroporation and spread onto LB plates containing the antibiotics gentamycin and rifampycin for selection of Agrobacteria and spectinomycin for selection of the expression vector (see Table 7 for application conditions of the antibiotics).

The LB plates were incubated at 28°C, 2X O/N. Resulting colonies were subjected to colony PCR using the same primers as used for the initial PCRs. Positive colonies were grown in liquid LB supplemented with gentamycin, rifampycin and spectinomycin at 28°C, 2X O/N. Glycerol stocks were made using the liquid culture and 30% glycerol in a 1:1 mixture and kept at -80°C.

2.2.11.3 Cloning of silencing constructs

For all PCRs the same template and enzymes were used as in section 2.2.11.2. Constructs to silence *AtAED9-1* and *AtAED9-2* (double silencing construct) as well as to silence *AtAED9-1*, *AtAED9-2*, and *AtAED9-3* (triple silencing construct) were designed according to (Wesley, Helliwell et al. 2001). All materials and methods used for RNAi silencing in transgenic plants were described by (Yin, Chory et al. 2005). The RNAi/hairpin-loop constructs generated to target *AtAED9-1* and *AtAED9-2* as well as *AtAED9-1*, *AtAED9-2*, and *AtAED9-3* for silencing are shown in Figure 9.

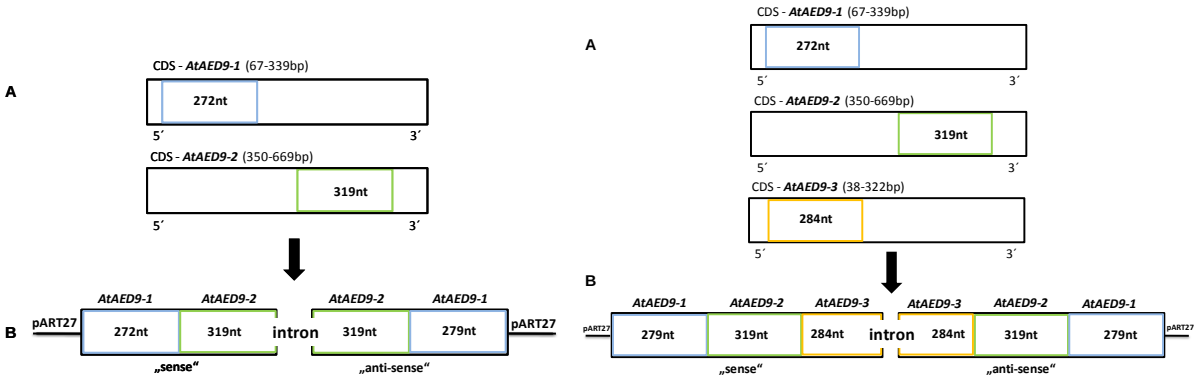


Figure 9: Basic schematic overview of the constructs used to silence *AtAED9-1* and *AtAED9-2* (on left) as well as *AtAED9-1, AtAED9-2*, and *AtAED9-3* (on right).

A: Parts of the coding sequence from *At*AED9-1 (blue box), *At*AED9-2 (green box) and *AtAED9-3* (yellow box) were selected **B:** Final silencing construct in pHANNIBAL– the intron between sense and anti-sense will form the loop of the hairpin that is formed by complementarity of the sense and anti-sense sequences

2.2.11.3.1 Generation of the double silencing construct

The double silencing construct was made by using the primer pairs HB-P17 and HB-P18 (parts of the coding sequence from *AtAED9-1*) and HB-P19 and HB-P20 (parts of the coding sequence from *AtAED9-2*) as shown in Table 27 for the initial PCR reactions. PCR was conducted with an annealing temperature of 59.5°C for both preparations. Both PCR products were applied to gel electrophoresis and the bands showing the correct fragment size (Table 27) were cut out of the gel. Subsequently, the PCR products were extracted using QIAquick® Gel Extraction Kit (Table 17). The thus purified PCR products were used as template for a second PCR with primer pair HB-P17 and HB-P20 ligating both templates into one larger DNA fragment. The PCR product was applied to gel electrophoresis and the band showing the correct fragement size of 598bp was isolated from the gel as described above (Table 17).

Figure 10 shows the cutting sites of the construct for subsequent cloning steps into pENTR™/D-TOPO® and pHANNIBAL. The purified PCR product was first cloned into pENTR™/D-TOPO® and sequenced as described above. Subsequently, the fragment was transferred to pHANNIBAL in the sense and anti-sense orientations.

Figure 10: Cutting sites of the double silencing construct.

The CACC sequence (in italics) is required for the entry of the fragment into pENTR™/D-TOPO®. The cutting sites for the construct in antisense direction are BamHI and HindIII. The cutting sites for the construct in sense direction are XhoI and KpnI.

pHANNIBAL and the double silencing construct in pENTR™/D-TOPO® were digested with *BamHI* and *HindIII* in order to ligate the double silencing construct into pHANNIBAL in the antisense direction. The products of the digestions were applied to gel electrophoresis and the bands showing the correct fragment sizes were extracted as described (Table 17). The digested pHANNIBAL vector and the digested double silencing construct were then ligated using T4 DNA ligase according to manufacturer´s recommendations. The resulting ligation product was transformed into *E. coli* (DH5α) as described above. Resulting colonies were screened by control digestion with *BamHI* and *HindIII*. A glycerol stock from E. coli harboring pHANNIBAL with the double silencing construct in antisense direction inserted was prepared as described above and kept at -80°C. The isolated pHANNIBAL plasmid containing the double silencing construct and the double silencing construct in pENTR™/D-TOPO® were digested with *XhoI* and *KpnI* in order to ligate the double silencing construct into pHANNIBAL in the sense direction. The products of the digestions were applied to gel electrophoresis and the bands showing the correct fragment sizes were extracted. These were ligated using T4 DNA ligase. The ligated construct was transformed into *E. coli* (DH5α). Resulting colonies were transferred to a liquid culture and grown at 37°C O/N. Plasmid DNA was isolated from the bacteria using the QIAprep® Spin Miniprep Kit (Table 17).

pHANNIBAL harboring the double silencing construct in antisense as well as the sense orientation was applied to control digestions. In these digestions the same restriction enzymes as in the cloning process were used: *BamHI* and *HindIII* for the construct inserted in the antisense direction and *XhoI* and *KpnI* for the construct inserted in the sense direction. Digestions were analyzed via gel electrophoresis.

Subsequently, pHANNIBAL harboring the double silencing construct in antisense as well as the sense direction and pART27 (binary vector/plant expression vector) were digested with *NotI* and separated via gel electrophoresis. Digested pART27 and the pHANNIBAL-double silencing construct (including CaMV35S, sense construct, intron, antisense construct and the OCS terminator) were ligated using T4 DNA ligase and transformed into *E. coli* (DH5α). Resulting colonies were screened by restriction with *NotI* and pART27 clones containing the double silencing construct were purified using the QIAprep® Spin Miniprep Kit (Table 17). The thus generated binary vector was transformed into *Agrobacterium tumefaciens* strain GV3101 via electroporation as described above and used for floral dipping of *Arabidopsis* (Clough and Bent 1998).

2.2.11.3.2 Generation of the triple silencing construct

The design of the triple silencing construct is shown in Figure 9. The detailed cloning steps were identical as carried out in the cloning procedure for the double silencing

construct. Hence, just the steps, which were not in accordance with the cloning procedure for the double silencing construct, are described.
Initial PCRs for the triple silencing construct were performed with the primer pairs HB-P21 and HB-P22 (parts of coding sequence from *At*AED9-1), HB-P23 and HB-P24 (parts of coding sequence from *At*AED9-2), and HB-P25 and HB-P26 (parts of coding sequence from *At*AED9-2) as shown in Table 27. PCR was conducted with an annealing temperature of 59.5°C for all preparations. The PCR products were applied to gel electrophoresis and the bands showing the correct fragment size (Table 27) were extracted. The thus purified PCR products were used as templates for the second PCR with primer pair HB-P21 and HB-P26 ligating all templates into the final 875 bp product that was used for subsequent cloning as described in the previous section.

2.2.11.3.3 Selection of transgenic plants

Seeds from plants subjected to floral dipping were surface sterilized as described above, taken up in sterile 0.1% top-agar, and spread on MS plates (Table 13) containing the antibiotics carbenicilin and cefotaxim (added to limit contamination on the plates) (Table 7). Transgenic plants (T1 generation) were selected by addition of hygromycin (for selection of over expression constructs) or kanamycin (for selection of RNAi constructs) to the plates (Table 7). The plates were kept at 4°C for 2 days and transferred to a plant growth chamber with light/dark cycles of 16 hour light/8 hour dark at 22°C. After two to three weeks plants carrying a transgene grew on selective plates, whereas plants not carrying a transgene died or were severely yellowed.
Approximately 30 surviving plants per transformation were transferred to soil and grown until seed harvest. Samples were taken from four to six week old T1 plants for selection of plants displaying good levels of over expression or silencing of the respective genes-of-interest. Plants expected to over express a gene were sprayed with 30 µM β-estradiol (Sigma, Taufkirchen, Germany) in 0.01% Tween20 (Calbiochem, an affiliate of Merck, Darmstadt, Germany), after which one leaf was harvested and subjected to protein expression analysis by Western Blot using αHA-antibodies (see below). One leaf per RNAi plant was harvested, leaf discs (Ø 8 mm) were stabbed out and incubated in a 0.5 mM SA-solution for 2 hours while shaking at 100rpm. After incubation the leaf discs were dried and frozen in liquid nitrogen. The samples were pestled and subjected to RNA isolation followed by RT-qPCR analysis of the expression of the RNAi target genes. Seeds from T1 plants displaying good over expression or silencing levels, respectively, were harvested, surface sterilized, and spread on selective plates as described above. The resulting T2 seedlings were selected further for a segregation ratio of 3:1 on the selective plates, indicating that the transgenic line carries only one copy of the transgene. Per thus selected transgenic line, eight seedlings were transferred to soil and grown until seed

harvest. Seeds from each individual T2 plant were surface sterilized and sown on selective medium for selection of homozygous lines in the resulting T3 generation. Each homozygous line was again tested for efficiency of over expression or silencing of the respective genes-of-interest allowing selection of two to three independent lines per transgene for use in further experiments.

2.2.12 Integrated proteomic approach

2.2.12.1 Determination of protein concentration

Determination of protein concentration was done with the Bradford Bio-Rad Protein Assay (Bio-Rad Laboratories GmbH, München, Germany) and carried out according to the manufacturer's instructions.

2.2.12.2 Apoplast extraction

Col-0 *DEX::AvrRpm1-HA* and *eds1-2 DEX::AvrRpm1-HA* plants were sown as a lawn. Four to five-week-old plants were sprayed until drop-off with 30 µM dexamethasone (DEX, Sigma, Taufkirchen, Germany) in 0.01% Tween20. Four to five hours later all above-ground tissue was harvested by cutting the plants right above the soil. Apoplast proteins were isolated in either APO-buffer I (used for LC-MS/MS analysis) or APO-buffer II (used for ICPL analysis) depending on subsequent analytical method (Table 11). Plant tissue in the appropriate buffer was exposed to a vacuum of up to 3 minutes in a normal vacuum chamber. Vacuum was kept for 10 minutes and afterwards slowly released. This procedure was repeated twice or, if necessary, three times. Infiltrated plants were carefully transferred to 20-30ml syringes hung in 50ml falcon tubes. The Falcon tubes with the inserted syringe were spun at 2250rpm at 4°C for 20 minutes. The flow-through was collected as the apoplastic liquid, which was kept frozen at -80°C.

2.2.12.3 LC-MS/MS analysis (liquid chromatography coupled with tandem mass spectrometry)

Apoplast extracts were concentrated on a 3kDA size exclusion column according to manufacturer's recommendation (Amicon® Ultra - Table 17). Remaining proteins were digested with trypsin (all trypsin digestions – in gel or in solution - were conducted at the Core Facility Proteomics, Helmholtz Zentrum Munich in the lab of Hakan Sarioglu) and further cleaned via a C18 reversed phase column (Table 17) (Rappsilber, Mann et al.

2007). The C18 column was initialized by rinsing two times with 500µl of 80% acetone (Merck, Darmstadt, Germany) and 5% formic acid (Merck, Darmstadt, Germany). Subsequently, the column was re-equilibrated with 500µl of 5% formic acid and loaded with the sample containing 100µg of trypsin-digested apoplast protein in 5% formic acid. The column was washed twice with 500µl of 5% formic acid, after which the peptides were eluted with 500µl of 70% acetone and 0.1% TFA (trifluoroacetic acid, Merck, Darmstadt, Germany).

The eluted peptide solution was speed-vac dried and subsequently analyzed by LC-MS/MS (HPLC-Orbitrap) as described below (Makarov 2000) (Hu, Noll et al. 2005). The resulting peptide sequences were compared to a common database (NCBI) by using Mascot as described below. Figure 11 summarizes the workflow from apoplast extraction via clean-up on a C18-column to final LC-MS/MS analysis of the protein samples.

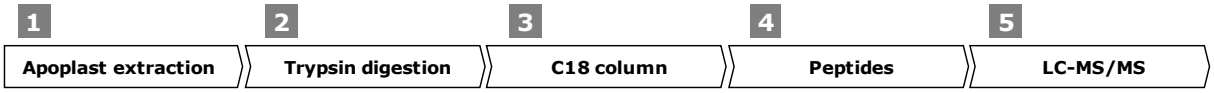


Figure 11: Workflow for analysis of the protein samples via LC-MS/MS.

Evaluation of the LC-MS/MS outcome was performed with Scaffold 3 Proteomics Software (Proteome Software, Inc., Portland, Oregon, USA).

2.2.12.4 ICPL (isotope coded protein labeling) analysis

Apoplast extracts were collected and concentrated on 3kDA size exclusion columns as above. ICPL works most efficiently between a protein content of 2,5 mg/ml and 5 mg/ml. In this study, 3,5 mg/ml per sample was used for analysis. The protocol below follows the ICPL kit of SERVA Electrophoresis; see kit/company guidelines for composition of buffers and solutions used. Figure 12 shows the experimental set-up of the ICPL process.

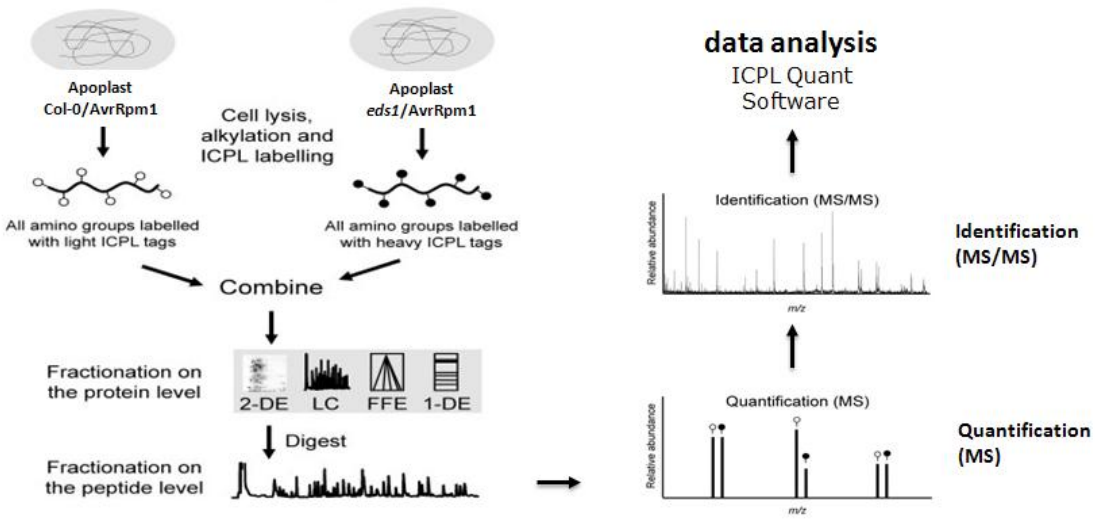


Figure 12: Experimental set-up of the ICPL analysis process

Figure modified based on (Kellermann 2008)

First, the samples with the appropriate protein content were dried (by speed-vac) and resuspended in 100µl of lysis buffer. A protein mixture was added to each of the samples as an internal control for subsequent analysis. Table 18 shows the different proteins and their quantitative ratio relative to each other in this internal control mixture. These proteins were dissolved in 20µl of lysis buffer and 6µl were added to each sample.

Table 18: Internal controls/standards used for ICPL.

Protein	Protein mix ICPL_0	Protein mix ICPL_6	Ratio ICPL_0/ICPL_6
BSA (bovine)	13.5µg	13.5µg	1:1
Ovalbumin (chicken)	24µg	6µg	4:1
Carbonic Anhydrase II (bovine)	12µg	24µg	1:2

After addition of 2,5µl of reduction solution the samples were incubated at 60°C for 60 minutes. Samples were cooled down to room temperature and shortly centrifuged. 2,5 µl of alkylation reagent was added to each sample and kept under light protection at 25°C for 30 minutes. In order to stop the reaction 2,5 µl of STOP solution 1 was added and samples were kept at 25°C for 15 minutes. The pH of the samples was adjusted to 8.3 $\pm$ 0.1 by addition of NaOH (2N) (Roth, Karlsruhe, Germany) or HCl (2N) (Merck, Darmstadt, Germany).

After the carbamidomethylation step described above the concentrated apoplast of Col-0 was labeled with 3µl of the light "ICPL_0" reagent (d0-N-nicotinoyloxy-succinimide, no heavy isotopes), whereas the apoplast of the *eds1-2* sample was labeled with 3µl of the

heavy "ICPL_6" reagent (d4-N-nicotinoyloxy-succinimide, containing 4 deuterium). Immediately after adding the labeling reagents the reaction tubes were filled with Argon to avoid oxidation. Moreover, the solutions were vortexed for 10 seconds, sonicated for 1 minute, and kept at 25°C for 2 hours. 10µl of stop solution was added to each sample and incubated at 25°C for 20 minutes to destroy excess reagent. After stopping the reaction, the two differentially labeled ICPL samples (Col-0 + ICPL_0 and *eds1-2* + ICPL_6) were combined and mixed thoroughly by vortexing.

By adding 2N NaOH the pH of the mixture was adjusted to 11.9 $\pm$ 0.1, which destroyed possible esterification products. After 20 minutes the same amount of 2N HCl was added to neutralize the sample. Purification of the labeled proteins was done by acetone precipitation with a subsequent protein fractionation on a 1D gel (see below). Precipitation was done by first adding the equal amount of dist. water to our sample amount and then a 5-fold excess (related to total volume of sample and water) of 100% ice-cold acetone. The sample was incubated at -20°C O/N. After incubation the precipitated proteins were spun down at 20000 g for 30 minutes at 4°C and supernatant was discarded. 200 µl of 80% ice-cold acetone was added to the pellet and shaken carefully. Another centrifugation step at 20000 g for 5 minutes at 4°C was performed and the supernatant was discarded. The samples were stored at -80°C before separation on a 1D gel.

2.2.12.5 MS analysis and data processing

For LC-MS/MS analysis, proteins were separated by using 1D gel electrophoresis in one repetition. For ICPL analysis 1D-gel separation was always applied. In all cases, gels were cut into 5 slices to reduce complexity of the samples. After coomassie staining and washing of the gel slices visible protein bands were excised and subjected to in-gel trypsin digestion as previously described (Sarioglu, Brandner et al. 2006). The digested peptides were separated by reversed phase chromatography (PepMap, 15 cm x 75 µm ID, 3 µm/100Å pore size, LC Packings) operated on a nano-HPLC (Ultimate 3000, Dionex) with a nonlinear 170 min gradient using 2% acetonitrile in 0.1% formic acid in water (A) and 0.1% formic acid in 98% acetonitrile (B) as eluents with a flow rate of 250 nl/min. The gradient settings were subsequently: 0-140 min: 2-40 % B, 140-150 min: 31-95% B, 151-160 min: Stay at 95% B. The nano-LC was connected to a linear quadrupole ion trap-Orbitrap (LTQ Orbitrap XL) mass spectrometer (ThermoFisher, Bremen, Germany) equipped with a nano-ESI source. The mass spectrometer was operated in the data-dependent mode to automatically switch between Orbitrap-MS and LTQ-MS/MS acquisition. Survey full scan MS spectra (from m/z 300 to 1500) were acquired in the Orbitrap with resolution R = 60,000 at m/z 400 (after accumulation to a target of 1,000,000 counts in the LTQ). The method used allowed sequential isolation of

the most intense ions, up to ten, depending on signal intensity, for fragmentation on the linear ion trap using collisionally induced dissociation at a target value of 100,000 ions. High resolution MS scans in the orbitrap and MS/MS scans in the linear ion trap were performed in parallel. Target peptides already selected for MS/MS were dynamically excluded for 30 seconds. General mass spectrometry conditions were: electrospray voltage, 1.25-1.4 kV; no sheath and auxiliary gas flow. Ion selection threshold was 500 counts for MS/MS, and an activation Q-value of 0.25 and activation time of 30 ms were also applied for MS/MS.

All MS/MS spectra were analyzed using Mascot (Matrix Science (Version: 2.2.06)). Mascot was set up to search the NCBI database, Num sequences for taxonomy: Arabidopsis thaliana (version from 19.04.2012 (64,961 sequences)) assuming the digestion enzyme trypsin and with a fragment ion mass tolerance (MS/MS) of 0.6 Da and a parent ion tolerance of 10 ppm (MS). Iodoacetamide derivative of cysteine as stable and oxidation of methionine, deamidation of arginine and glutamine as variable modifications were specified in Mascot as variable modifications.
For LC-MS/MS analysis Scaffold (version Scaffold_3_00_07, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 80.0% probability as specified by the Peptide Prophet algorithm (Keller, Nesvizhskii et al. 2002). Protein identifications were accepted if they could be established at greater than 95.0% probability and contained at least 2 unique peptides. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.

Data processing and identification for ICPL analysis was done according to (Gaupels, Sarioglu et al. 2012) with one following exception: Protein identification by at least two unique peptides in all three biological replicates were taken into consideration.

2.2.13 SAR experiments

In order to evaluate the role of candidate signaling proteins in SAR, SAR experiments were conducted in wt and mutant plants (Figure 13). For each inoculation the appropriate *P. syringae* strain was grown O/N on NYGA medium with antibiotics (Table 2 and Table 7/Table 10 for bacteria and antibiotics/media). On the day of the inoculation, cells were removed from the plates and resuspended in 10 mM $MgCl_2$, pH 7.0. *Pst AvrRpm1* was diluted to yield a suspension containing 1 x 10^6 CFU/ml. This was achieved by measuring the optical density of the undiluted suspension at a wavelength of 600nm using a photometer (Ultrospec 3100 pro, GE Healthcare, Munich, Germany). The required dilution

was calculated assuming the suspension held 10^8 CFU/ml of bacteria when OD600 = 0.2. Two lower (1°) leaves of 4-week-old *Arabidopsis* plants were infiltrated with the suspension containing 1 x 10^6 CFU/ml of *Pst AvrRpm1* or with 10 mM $MgCl_2$ as a control. Two or three days after the primary treatment, upper (2°) leaves were infiltrated by a needleless syringe with 1 x 10^5 CFU/ml of *Pseudomonas syringae* pv. *tomato* (DC3000). After another four days, systemic leaves were harvested and growth of bacteria was analyzed.

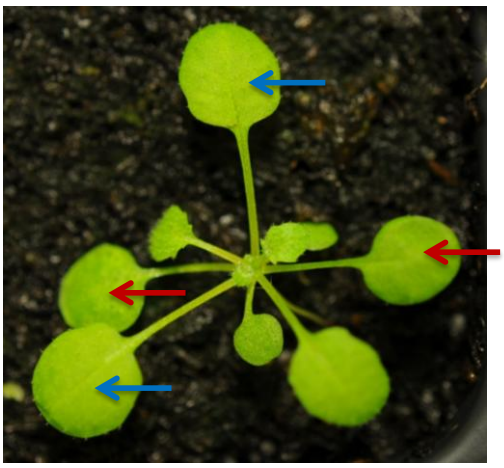


Figure 13: SAR induction.

Three genotypes were used: wt, *aed9-1* and *aed9-3*; 1° leaves (red arrows) of the different genotypes were infiltrated with *Pst AvrRpm1* or with 10 mM $MgCl_2$ as a control. After 3 days 2° leaves (systemic, blue arrows) were infiltrated with DC3000. After another 4 days the 2° infected leaves were harvested.

For analysis of DC3000 growth in leaves, bacteria were extracted from three 6 mm leaf discs per sample (in triplicate per genotype and treatment). The discs were shaken at 600 rpm at 25 °C for 1 hour in 500 µL 10 mM $MgCl_2$ with 0,01% Silwet. The resulting bacterial suspension was diluted in 10 mM $MgCl_2$ in five serial 10X steps. Subsequently, 20 µl of each dilution was pipetted (or 'spotted') onto NYGA plates with antibiotics (see above) and grown for two days at 28 °C.

2.2.14 Growth curve analysis of Pseudomonas syringae pv. *tomato* (*Pst*) AvrRpm1, *Pst* AvrRps4 and DC3000

The interaction of *aed9-1* and *aed9-3* with three different *Pst* strains is analyzed by performing bacterial growth curve experiments.

For each inoculation the appropriate *Pst* strain was grown O/N at 28°C on NYGA medium with antibiotics. On the day of the inoculation, cells were removed from the plates and resuspended in 10 mM $MgCl_2$, pH 7.0. The different *Pst* strains were diluted to yield a suspension containing 1 x 10^5 CFU/ml. Each *Pst* strain was infiltrated into 4-week-old wt, *aed9-1*, and *aed9-3 Arabidopsis* plants with a density of 10^5 CFU/ml in 10mM $MgCl_2$. Two hours after infiltration the first samples from the infiltrated leaves were taken. This time point was taken as t=0. The following additional time points were harvested: 2 hours, 1,

2,3 and 6 days after infection. For analysis of *Pst* growth in leaves, bacteria were extracted from three 6 mm leaf discs per sample (in triplicate per genotype). Before punching out the leaf discs, the surface of the leaf area was sterilized by dipping them for 10s into 70% ethanol followed by 10s in bidest H_2O. The analysis of bacterial growth in leaves was done as described above.

2.2.15 Gene expression analysis of candidate genes during a SAR experiment as well as after infection with different *Pst*-strains

In order to evaluate the behavior of certain genes under different conditions, qPCR analyses were conducted during a SAR experiment as well as after infection.

Samples were harvested during infection experiments from both local, infected (1°) and systemic (2°) tissue. RNA was isolated as described above and cDNA generated by using SuperscriptII reverse transcriptase (Table 6). Subsequently, gene expression was analyzed by qPCR (Table y for SYBR Green etc.) using gene-specific primers listed in Table 10.

2.2.16 1D - SDS-Page and western blot analysis

A 1D-SDS-PAGE (one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was used to separate proteins, which were subsequently used for LC-MS/MS analyses or transferred to a PVDF transfer membrane with 0.45µm pore size (Amersham Hybond™-P, GE Healthcare, Freiburg, Germany) by electroblotting. For Western blot analysis, the membrane-bound proteins were challenged with a primary antibody, followed by a secondary antibody coupled to an enzyme (horseradish peroxidase) whose activity caused the deposition of a chemiluminescent product. All antibodies and solutions used for Western blot analysis are described in Table 8 and Table 12.

Proteins were separated according to their molecular weight with 10% or 15% resolving gels and 4% glycine-SDS-polyacrylamide-stacking-gels with the Mini-PROTEAN® Tetra Cell from Bio-Rad (München, Germany). Gels were prepared as described by Sambrook and Russell (Molecular cloning: a laboratory manual, 2001) according to Laemmli (1970). Protein extracts were mixed 1:1 with 2x reducing sample buffer, heated for 5 min at 95°C, and loaded into the gel pockets. An electric tension of 120V was applied until the tracking dye entered the resolving gel, after which the electric tension was increased to 150V until the tracking dye reached the bottom of the resolving gel.

For Western blot analysis, proteins were transferred from polyacrylamide gels to Hybond™-P PVDF membranes in wet conditions using the Mini-PROTEAN® Tetra Cell

from Bio-Rad (München, Germany). For wet transfer, the gel and membrane were sandwiched between sponge and paper (paper/gel/membrane/paper/sponge) and all were clamped tightly together after ensuring no air bubbles had formed between gel and membrane. The sandwich was submerged in transfer buffer to which an electrical field of 250mA was applied for 2 hours. After transferring the proteins to the membrane, further protein binding was blocked by carefully shaking the membrane for one hour in 2% milk powder dissolved in 1X PBS buffer. Subsequently, 16 µl of the first antibody (usually αHA, see Table 8) solved in 10 ml blocking buffer was applied to the membrane. The membrane was incubated with the first antibody at 4°C O/N under slight shaking. The membrane was then washed with 1X PBS supplemented with 0.05% Tween20 four times for 15 minutes each, followed by a one hour incubation with a 10 ml 1X PBS + 0.05% Tween20 solution containing 4 µl of the secondary antibody (Table 8). Subsequent to 4 washing steps (as above) with 1X PBS, a 1 ml mixture of the two components (Luminol/Enhancer solution and Peroxide solution) from the Immun-Star™ WesternC™ Kit (Bio-Rad, München, Germany) were added to the membrane. Chemiluminescence was visualized using a Typhoon TRIO+ Variable Mode Imager (Amersham Biosciences, Freiburg, Germany).

2.2.17 Transient over-expression of proteins-of-interest in *Nicotiana tabacum* coupled with tobacco mosaic virus (TMV) infection

2.2.17.1 Transient over-expression of proteins-of-interest

Agrobacterium tumefaciens strain GV3101 carrying different expression vectors (*AtAED*-genes in pER8-GW-Cterm-3XHAStrep) was infiltrated into tobacco leaves using a syringe without needle (´agroinfiltration´ (Fischer, Vaquero-Martin et al. 1999)). Agroinfiltration exploits the delivery of Agrobacteria into intact leaf tissue. Once present in the leaf, bacterial proteins catalyze the transfer of the gene-of-interest into the host cells. The bacteria harbored plasmids containing the different *AtAEDs* between an estradiol-inducible promoter and a C-terminal HA-Strep-tag. All solutions used in this section are described in Table 15.

A. tumefaciens was grown at 28 °C (Shaker for cultures, Brunswick G25, New Jersey, USA) for two days in LB medium with selective antibiotics: rifampycin, gentamycin and spectinomycin (for selection of the transgene-containing plasmid). Cells were diluted 1:100 in minA medium supplemented with 10mM MES, 20 µM acetosyringone, and appropriate antibiotics. Bacteria were grown for another day at 28 °C and harvested by centrifugation. Subsequently, the bacteria were resuspended in MMA and incubated at

room temperature for three hours without shaking. For infiltration six-week-old *Nicotiana tabacum* (tobacco) plants were used and three leaves per plant were infiltrated with the bacterial suspension in MMA. 24 Hours later the infiltrated leaves were sprayed with 30µM estradiol in 0.01% Tween-20 to induce expression of the transgenes. Samples were taken 60 hours after infiltration and 40 hours after estradiol spray. Samples consisting of three leaf discs with 8mm diameter were ground under liquid nitrogen, taken up in 125 µl of 2 X SDS protein gel loading buffer (Table 12), and used for Western blot analysis.

2.2.17.2 Systemic infection with TMV subsequent to transient over-expression of proteins-of-interest in the local tissue

The systemic leaves (2°) were infected with TMV. This was done 7 days after agro infiltration of the local leaves (1°). A TMV virus stock isolated from inoculated leaves of *N. tabacum cv.* Xanthi was used for all experiments. TMV infection was done by rubbing a dilution of 1:500 of virus in phosphate buffer (Table 3) with siliciumcarbid (Sigma, Deisenhofen, Germany) onto the leaves. TMV lesions were photographed and/or measured 7 days after infection. Figure 14 illustrates the different treatments on the local/agro infiltrated tissue (1°).

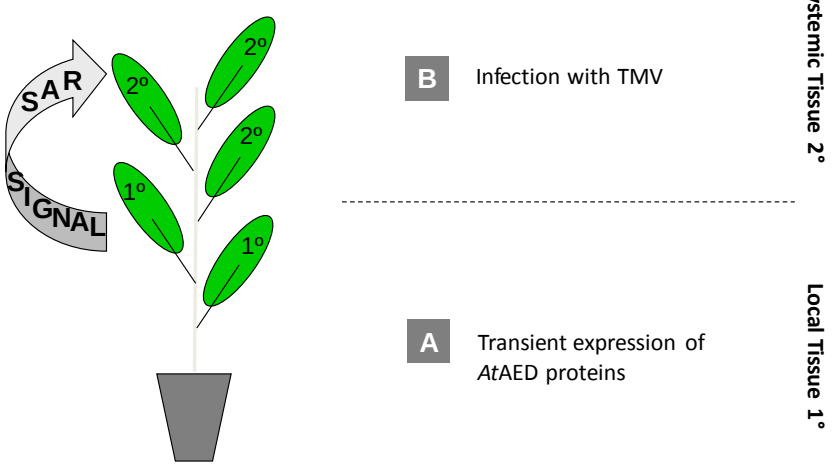


Figure 14: Summary of different treatments of *Nicotiana tabacum* in either the primary (1°) or the systemic tissue (2°)

A) Western blot of locally (1°) agro-infiltrated *Nicotiana tabacum* tissue 60 hrs after infiltration B) Infection of the systemic tissue (2°) with TMV 7 d after agro infiltration.

Chapter 3: RESULTS

3.1 Protein analysis: 2D-gel, LC-MS/MS and ICPL

Protein accumulation was analyzed in the apoplast of *AvrRpm1*-expressing Col-0 wt and *eds1-2* mutant plants. In order to cover the largest possible range of proteins three different proteomic approaches were chosen to analyze the samples. These methods were 2D-gel (two-dimensional gel) (see preliminary data in Introduction), LC-MS/MS and ICPL analyses. The 2D-gel and LC-MS/MS approaches resulted in qualitative or at least semi-quantitative information about proteins either present or missing in one sample as compared to the other. ICPL uses stable labeling of proteins in different extracts with d0-/d4-N-nicotinoyloxy-succinimide, or light and heavy isotopes, respectively. This approach yielded quantitative information regarding the relative amount of specific proteins present in both samples. Together, the three proteomics approaches resulted in a list of 21 apoplastic, *EDS1*-dependent (AED) proteins. The following paragraphs describe the results gained from the integrated proteomics approach.

3.1.1 Protein analysis via 2D-gel comparison

A classical method to analyze proteins in differently treated samples is the 2D-gel analysis. 2D-gel analyses were and are still widely applied in protein research. Vlot *et al*. conducted a 2D-gel analysis at the Max Planck Institute for Plant Breeding Research, Cologne, Germany to compare proteins accumulating in the apoplast of Col-0 *DEX::AvrRpm1-HA* and *eds1-2 DEX::AvrRpm1-HA* plants after induction of the transgene with DEX (unpublished). Table 29 in the supplement gives an overview of the AED proteins detected via this approach. The protein name, acronym, locus number of the respective gene as well as the predicted protein size in kDA is shown.

3.1.2 Protein analysis via LC-MS/MS

The LC-MS/MS technique is becoming increasingly important in biomolecule analysis. A mass spectrometer measures the mass-to-change ratio (*m/z*) of gas-phase ions. In a typical proteomics experiment, the sample is delivered to the mass spectrometer via a chromatographic device, in our case a HPLC column, and ionized and vaporized in the ion-source. The resultant ions are sorted by their *m/z* in the mass analyzer. We used an LTQ Orbitrap XL (a linear quadrupole ion-trap Orbitrap), which contains an electrospray ionization source (nano-ESI source) to ionize samples. During the first MS step, peptides

of trypsin-digested proteins are ionized, which results in the formation of charged particles. These ions are separated according to their mass-to-change-ratio by an electromagnetic field and finally detected. Subsequently, peptides are isolated based on their *m/z* and ionized a second time. The mass spectra of the resultant mixture of smaller peptides allow deduction of the amino acid sequence of the original peptide by comparing the masses of the peptides of the unknown protein to a peptide mass database.
Before proteins in apoplast extracts from *AvrRpm1*-expressing wt and *eds1-2* mutant plants could be analyzed by LC-MS/MS the samples were cleaned by solid phase extraction (SPE) to avoid clogging of the HPLC column. Two approaches were considered for SPE clean up of the protein samples; these are illustrated in Figure 15.

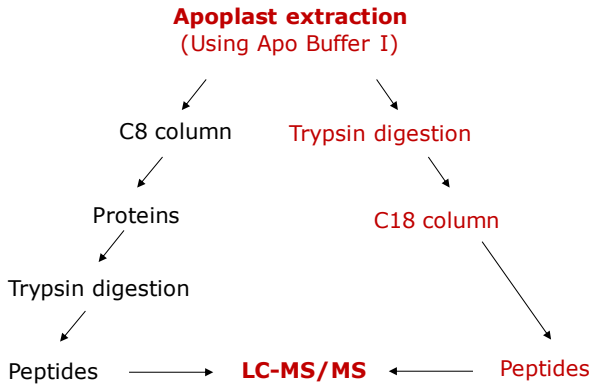


Figure 15: Overview of the cleaning steps before LC-MS/MS analysis via SPE.

After the extraction of the apoplast the samples were either digested with trypsin and cleaned on a C18 column or cleaned first on a C8 column and then digested with trypsin (highlighted in red – approach finally used to clean the samples).

For SPE C8 columns are more suitable for proteins, whereas C18 columns are more commonly used for peptide samples (Rappsilber, Mann et al. 2007). Both columns were tested in this study with the C18 column showing a much higher protein recovery rate as compared to the C8 column. Therefore, protein samples were digested with trypsin and then cleaned on C18 columns before LC-MS/MS analysis. On average we identified around 350 proteins per sample. The relatively low number of proteins is mainly caused by the enormous complexity of the samples and the limits of protein detection of the Orbitrap technique.

For the last set of LC-MS/MS runs one protein extract each from wt and mutant plants was subjected to 1D gel fractionation before further analysis. 1D-gel fractionation is a widely applied method to reduce the complexity of protein samples prior to LC-MS/MS analysis. In this study, gels were cut into five slices per protein sample that were each subjected to in-gel trypsin digestion and subsequent analysis by LC-MS/MS. After 1D gel fractionation we were able to detect a total number of 1150 proteins per sample. Here it needs to be noted that the apoplast extraction method, which was used in this study, is

the best method currently available, but allows varying amounts of cytosolic leakage when samples are extracted from *Arabidopsis thaliana*. Therefore, apoplastic proteins were enriched in the extracts analyzed here, while some proteins originating from the cytosol also were detected.

In up to three out of five independent sets of LC-MS/MS runs three proteins, which accumulate in the apoplast of *AvrRpm1*-expressing *Arabidopsis* wildtype, but not *eds1* mutant plants, were detected (Table 19). To this end, the Scaffold 3 software had been used to analyze the different LC-MS/MS runs. Stringent filtering methods were applied. The minimum number of identified peptides per protein was set at two. This means that two unique peptides must be detected from one protein to consider the protein to be identified. Moreover, the minimum protein ID probability was set at 95.0%.

Due to the enormous complexity of our protein samples, the relative appearance of the identified proteins differed in the different runs or sample sets. Glycosyl Hydrolase Family 3 protein (*At3G19620* – *At*AED8) accumulated differentially in the samples of two sets as did Glutamate Synthase 1 (*At5G04140* – *At*AED10). Legume Lectin Family Protein (*At3G15356* – *At*AED9-1) accumulated differentially in the two samples of three sets; in one set the difference was present (wt) versus not present (*eds1*) and two times the difference was semi-quantitative. The term semi-quantitative in this context refers to the protein identification probability being over 95% in *Arabidopsis* wild type extracts and between 20 and 49% in the *eds1-2* extracts. These three proteins were added to the list of AEDs and numbered accordingly. A BLAST sequence similarity search of *AtAED9-1* revealed that *AtAED9-2* is a very close homologue, with a similarity to *AtAED9-1* of 89%. Therefore, it was included in *in planta* studies of *AtAED9* family members below.

Table 19: Overview of AED proteins identified using LC-MS/MS.

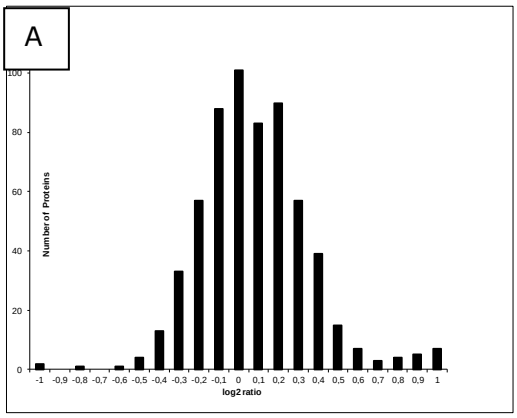
Protein	Acronym	Locus number	Predicted size [kDa]
Glycosyl hydrolase family 3 protein	*At*AED8	*At3G19620*	*85.4*
Legume lectin family protein	*At*AED9-1	*At3G15356*	*29.7*
Lectin-like protein (89% homologues gene to *At*AED9-1)	*At*AED9-2	*At3G16530*	*30.5*
Glutamate synthase 1	*At*AED10	*At5G04140*	*17.9*

3.1.3 Protein analysis via ICPL

ICPL is a quantitative proteomic method based on isotope labeling of all free amino acid groups in proteins. It is the only method, where the labeling step is already performed on the protein level. After labeling of apoplast extracts from *AvrRpm1*-expressing wt and *eds1* mutant plants, a so-called 'duplexed' ICPL was set up comprised of two different proteome states. The differently labeled 'states' wt and mutant were combined in one sample and the complexity of the sample was reduced by 1D-gel separation. Gels were divided into five slices that were subjected to trypsin digestion followed by LC-MS/MS analysis. The quantification of this ICPL experiment was facilitated by the ICPL*Quant* software (Brunner, Keidel et al. 2010). One heavy/light count refers to one peptide pair differentially labeled and therefore countable.

The data shown in supplementary table Table 30 are the result of three independent ICPL runs. Statistics were conducted to evaluate which proteins were significantly regulated in an *EDS1*-dependent manner. To this end, stringent filtering methods were applied to analyze each run. Two criteria for peptide filtering were applied: a peptide score of 18 and a maximum peptide rank of 3. A peptide score of 18 was used as a parameter for identification of proteins derived from database searches (Chepanoske, Richardson et al. 2005). Peptide rank of 3 refers to statistical evaluation of the MS/MS spectra in relation to the theoretical masses of the peptides. The software gives several recommendations for every MS/MS spectra and the related peptide sequence, which are the most probable ones. These probabilities were compared with each other and the top 3 were taken into account. Hence, two out of the three probabilities are incorrect, but their scores are so similar that the top 3 probabilites are further processed and evaluated on the protein level. The proteins were filtered according to the minimum number of peptides identified. Here, the threshold was set at 2 peptides. Figure 16 shows the statistical data and the lognormal distribution of the ICPL experiment.

A



B	Lognormal distribution
n	610
μ	1,081073194
stdev (sigma)	0,344211847

Figure 16: Statistical data from the three independent ICPL runs.
A: Distribution of all detected proteins based on the log2 ratio averaged after three ICPL runs B: Lognormal distribution averaged after three ICPL runs

Figure 16A shows the distribution of all n=610 detected proteins. The y-axis indicates the number of proteins, whereas the x-axis shows the log2-ratio of these proteins relative to each other in wt and *eds1-2*-derived samples. Consequently, the figure illustrates how many proteins follow a certain regulation based on their heavy/light ratio across the three ICPL runs performed. In Figure 16B key facts about the lognormal distribution are shown. Averaged on the ICPL-runs the sample size was 610 proteins (n), the average level of regulation was approximately 1.08 (protein median) with a standard deviation of approximately 0.34. Peptide quantification was evaluated with ICPL Quant Software (Brunner, Keidel et al. 2010), which automatically calculates the peptide pair ratios by comparing their relative signal. The protein identities were established with the same software using NCBI as a database.

To validate each ICPL run the protein samples were spiked with known concentrations of control proteins. Table 20 shows the ICPL result of these control proteins across the three independent ICPL runs performed in this work. These proteins represent the standard/internal control for the ICPL analysis, because their heavy/light ratio was known.

Table 20: Spiked proteins used as standards/internal controls in the ICPL analysis.

Accession UniProt	Protein name	Σ# Heavy/Light counts	Heavy/Light ratio measured	Heavy/Light variability [%]	Heavy/Light theoretical
CAH2_BOVIN	Carbonic anhydrase II	18	2.43	9.8	2,0
ALBU_BOVIN	Serum albumin	38	1.049	9.7	1,0
OVAL_CHICK	Ovalbumin	50	0.242	18.1	0,25

The three proteins spiked into the samples before analysis were carbonic anhydrase II, serum albumin and ovalbumin. In the three ICPL runs all control proteins showed different values in the number of their heavy/light counts, indicating a certain heavy/light variability between the runs. The measured heavy/light ratio of serum albumin and ovalbumin were close to the respective expected values deviating from the expected values only by roughly 3-5%. However, the measured heavy/light ratio of carbonic anhydrase II deviated from the expected value by 21.5%, with a measured value of 2.43 against an expected value of 2.0. All three proteins were pre-mixed in one reation tube as part of the duplex ICPL-Kit (Table 17). Hence, it is difficult to explain why the heavy/light ratio for one out of three proteins deviated from the expected value. Perhaps the amount of carbonic anhydrase II was inaccurate or this protein was not stable, which would explain the relatively high aberration compared to the other two proteins.
Taken together, the ICPL measurement of the internal protein controls yielded the theoretically expected values for two out of three proteins and therefore the ICPL runs were scored as valid. In total 759 protein IDs were detected, from which 610 were labeled and therefore quantifiable.

10 proteins accumulated to a significantly higher level in the apoplast of *avrRpm1*-expressing wt as compared to the apoplast of *avrRpm1*-expressing *eds1* mutant plants. The wt:eds1 ratio of these 10 proteins varied from 5.61 to 1.96. Table 21 shows the statistically significant, regulated proteins with their locus number, their normalized heavy/light ratio (regulation) and their heavy/light counts after three biological repetitions/runs. In this case, normalized ratio refers to the regulation of the proteins, which is normalized to the distribution of the ratios, meaning normalized to the average value of ~1.08 (protein median).
It should be noted that the proteins listed in Table 21 cannot be found among the AED proteins found in the LC-MS/MS analysis. This may be due to the necessity for some level of accumulation of corresponding peptides in both plant genotypes in the case of ICPL. Proteins/peptides that do not occur in one or the other sample, e.g. in the *eds1-2* mutant, are not detected by ICPL and therefore not quantified.

Table 21: 10 proteins were significantly regulated in an *EDS1*-dependent manner after ICPL runs of three biologically independent sample set repetitions.

Protein	Locus-Number	Normalized heavy/light ratio	Heavy/light counts (run1; run2; run3)
PR2 (pathogenesis related protein 2)	*At3G57260*	5.61	3;1;1
receptor serine/threonine kinase-like protein	*At4G18250*	2.92	1;1;1
PR5 (pathogenesis related protein 5)	*At1G75040*	2.90	4;4;6
hypothetical protein	*At2G18660*	2.65	2;2;1
chitinase, putative	*At2G43570*	2.33	2;3;1
pectin methylesterase like protein	*At3G14310*	2.31	9;12;5
ATGSTF2 (glutathione S-transferase PHI2)	*At4G02520*	2.27	3;2;2
NIT1; indole-3-acetonitrile nitrilase/ indole-3-acetonitrile nitrile hydratase/ nitrilase	*At3G44310*	2.24	17;10;5
legume lectin family protein	*At5G03350*	2.00	4;5;4
EP1; protein kinase	*At4G23170*	1.96	2;2;1

PR2, or beta-1,3-glucanase 2, shows the highest normalized heavy/light ratio of 5.61. This means that PR2 accumulates to a 5.61-fold higher level in the apoplast of *AvrRpm1*-expressing Col-0 as compared to *eds1-2*. Focusing on the heavy/light counts, PR2 had three countable peptide pairs in the first run, whereas one peptide pair was counted in run 2 and 3 each.

The ICPL-derived list of potentially SAR-inducing proteins (Table 22) contains a member of the lectin-like protein family, *At*AED9-3. The legume lectin family protein shows a regulation of 2.00 and is countable in three different ICPL runs (countable peptide pairs 4;5;4). This protein is similar to *At*AED9-1, found in the LC-MS/MS analysis and its closest homologue *At*AED9-2. The relationship between *At*AED9-1, *At*AED9-2 and *At*AED9-3 and their placement within the legume lectin protein family is described in more detail below.

Table 22: Overview of the AED proteins detected by ICPL.

Protein	Acronym	Locus number	Protein size [kDa]
legume lectin family protein	*At*AED9-3	*At5G03350*	*30.1*
PR2 (*pathogenesis related protein 2*)	*At*AED11	*At3G57260*	*37.3*
receptor serine/threonine kinase-like protein	*At*AED12	*At4G18250*	*95.2*
PR5 (*pathogenesis related protein 5*)	*At*AED13	*At1G75040*	*25.2*
hypothetical protein	*At*AED14	*At2G18660*	*14.5*
chitinase, putative	*At*AED15	*At2G43570*	*29.7*
pectin methylesterase like protein	*At*AED16	*At3G14310*	*64.2*
ATGSTF2 (glutathione S-transferase PHI2)	*At*AED17	*At4G02520*	*24.1*
NIT1; indole-3-acetonitrile nitrile hydratase/nitrilase	*At*AED18	*At3G44310*	*38.1*
EP1; protein kinase	*At*AED19	*At4G23170*	*29.7*

ICPL was the proteomic method, which yielded the highest number of proteins regulated in *AvrRpm1*-expressing *Arabidopsis* Col-0 plants in an *EDS1*-dependent manner.

3.1.4 Summary of the proteomic analyses

Figure 17 provides an overview of the complete proteomic workflow including the methods used, the number of proteins detected by each method, and the final result. 21 AED proteins were identified that accumulated differentially in the apoplast of *AvrRpm1*-expressing Col-0 plants as compared to *AvrRpm1*-expressing *eds1-2* mutant plants.

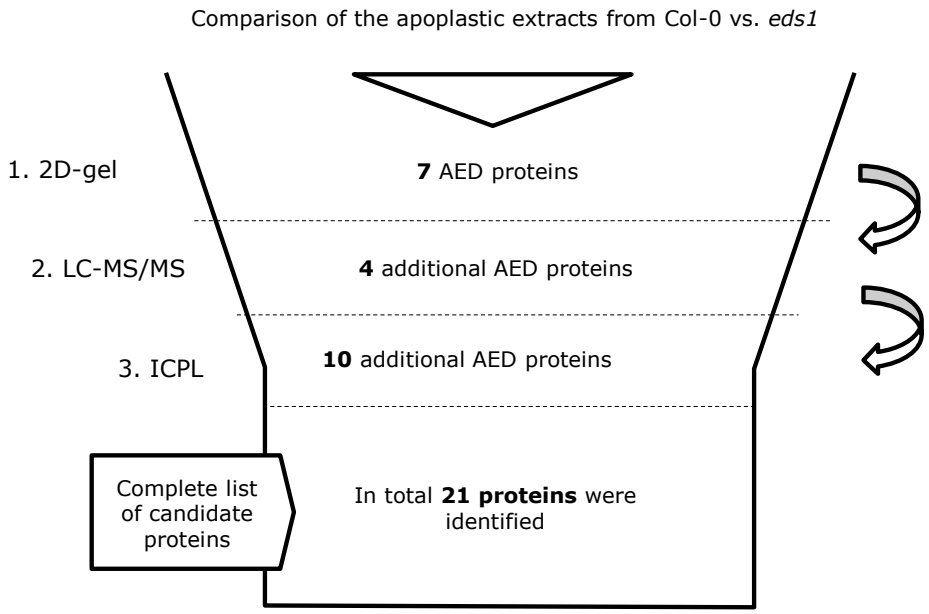


Figure 17: Summarized workflow of the complete proteomic approach.

In the figure the proteomic workflow is illustrated on the left side, whereas the number of proteins identified using the different methods is shown in the center part.

3.2 *AED* gene expression after infection of *Arabidopsis* with *P. syringae AvrRpm1*, *P. syringae AvrRps4*, or *P. syringae*.

A classical way to analyze the expression of (a) gene(s) under different environmental stimuli is the quantitative reverse transcriptase polymerase chain reaction, shortly named qRT-PCR.

The expression was evaluated of the genes encoding the AED proteins detected above upon infection of wild type and *eds1-2* mutant plants with three different strains of *P. syringae* pathovar *tomato* (referred to in this work as *Pst* – strain is also known as DC3000). To this end, qRT-PCR measurements were conducted after *Arabidopsis* wild type and *eds1-2* plants were treated with *Pst AvrRpm1*, *Pst AvrRps4,* or *Pst*. The expression of *AtAED19* was not analyzed due to high similarity with other genes (*At4G23140, At4G23150* and *At4G23160*). Consequently, no specific primer pair for *AtAED19* could be designed.

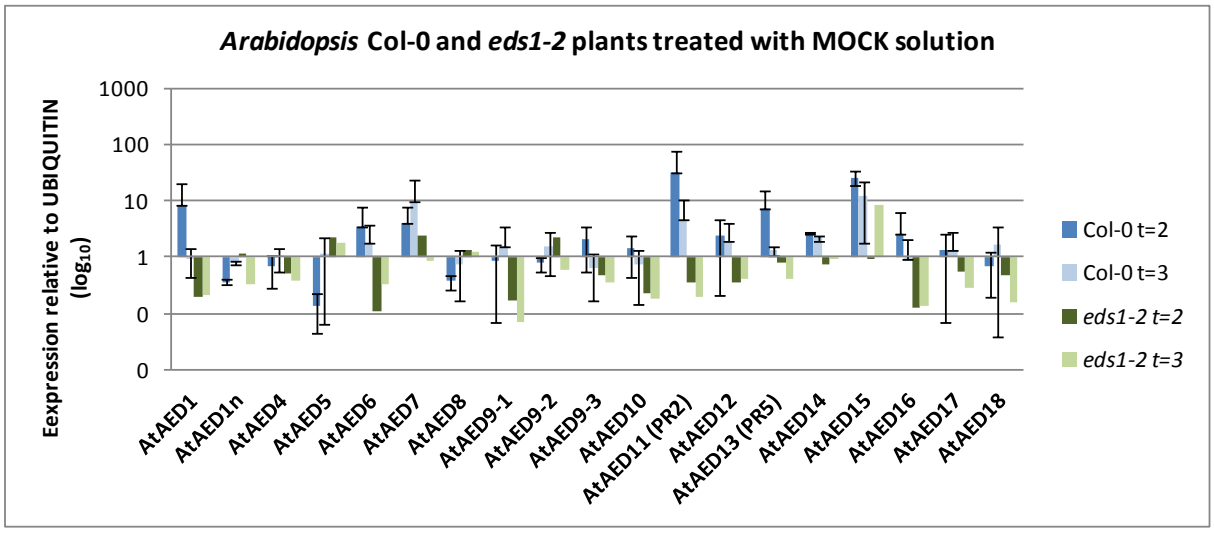


Figure 18: Arabidopsis Col-0 wild-type and *eds1-2* plants treated with MOCK solution (10mM $MgCl_2$). Samples were taken two and three days after infiltration.

Experiments were repeated two times in Col-0 wild type plants with comparable results. The analysis for the *eds1-2* plants was conducted once (no standard deviation could be shown).

Figure 18 shows the gene expression of the *AED* genes after treatment of Arabidopsis leaves with MOCK-solution (10mM $MgCl_2$) two and three days after treatment. Expression is shown relative to that in untreated plants of each genotype (wt and *eds1-2* mutant, respectively). The blue bars represent the gene expression in the wild type plants at the two different time points, whereas the green bars display the expression in the *eds1-2* plants. Among the genes are some that are up-regulated in the wild type plants, e.g. *AtAED11* (*PR2*) and *AtAED15*. This can potentially be related to wounding while infiltrating the MOCK solution. Other genes are down-regulated and some are not regulated. In the case of the *eds1-2* plants most of the genes are down-regulated or not regulated.

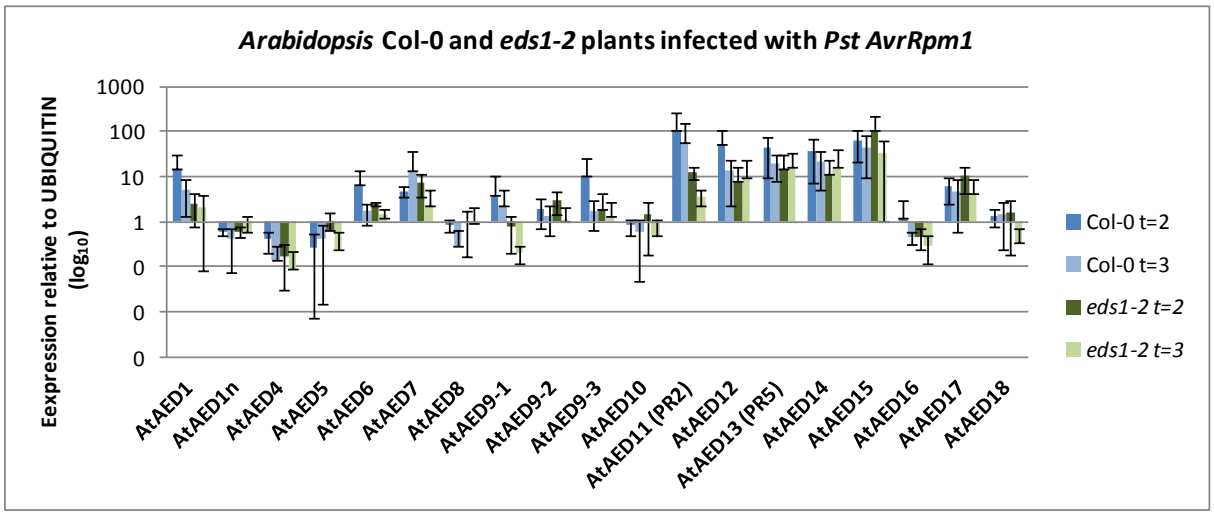


Figure 19: Arabidopsis Col-0 wild-type and *eds1-2* plants treated with *Pst AvrRpm1*. Samples were taken two and three days after infiltration.

Experiments were repeated three times in Col-0 wild type plants with comparable results. The analysis for the *eds1-2* plants was conducted twice with comparable results.

The regulation of the *AED* genes after infection of wild type and *eds1-2* plants with *Pst AvrRpm1* is shown in Figure 19. Expression is shown relative to that in untreated plants of the same genotypes. Especially expression of the cluster of genes *AtAED11* (*PR2*) through *AtAED15* as well as *AtAED7* is upregulated in wild-type as well as in *eds1-2* plants, indicating that these genes are induced downstream from AvrRpm1 independently of *EDS1*. *AtAED1* is up-regulated in wild type plants, but not in the *eds1-2* mutant, indicating that expression of *AtAED1* is induced by *PstAvrRpm1* in an *EDS1*-dependent manner, even if *AtAED1* as well as *AtAED11* and *AtAED15* are showing an up regulation after MOCK-treatment.

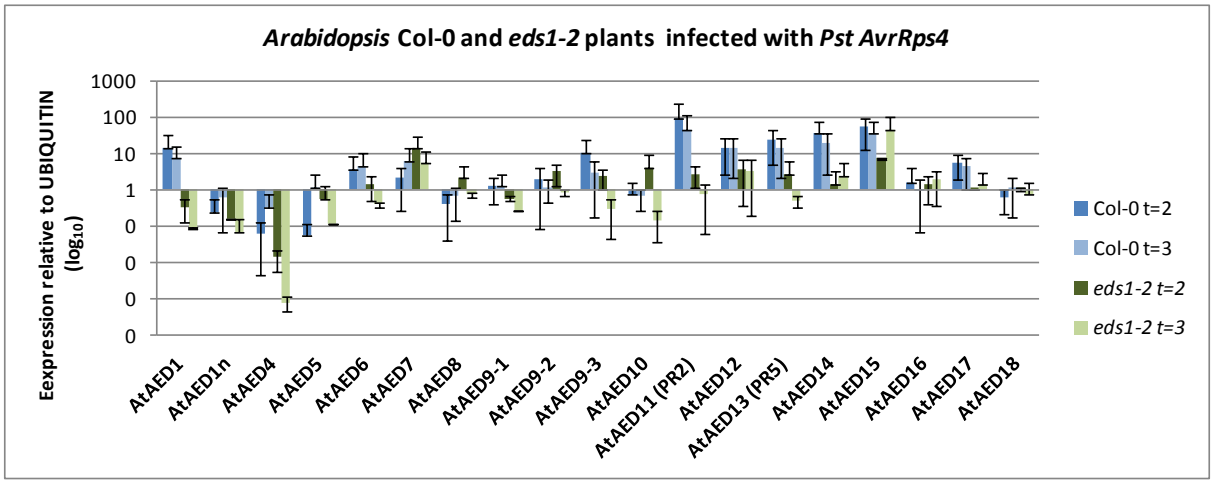


Figure 20: Arabidopsis Col-0 wild-type and *eds1-2* plants treated with *Pst* AvrRps4. Samples were taken two and three days after infiltration.

Experiments were repeated three times in Col-0 wild type plants with comparable results. The analysis for the *eds1-2* plants was conducted twice with comparable results.

The regulation of the *AED* genes after infection of wild type and *eds1-2* plants with *Pst AvrRps4* is shown in Figure 20. Again, expression of some genes is up-regulated in wild type plants at both time points, e.g. *AtAED1* and *AtAED11* (*PR2*). Expression of other genes is down-regulated in wild type as well as in *eds1-2* plants, e.g. *AtAED4* and *AtAED5*. *AtAED16* and *AtAED18* are examples of non-regulated genes. Furhtermore, the cluster of genes *AtAED11* (*PR2*) through *AtAED14* displays an up-regulation in the wild type *Arabidopsis* plants and no regulation in the *eds1-2* mutant plants, indicating that they are induced in an *EDS1*-dependent manner downstream from AvrRps4. *AtAED7* and *AtAED15* both are up-regulated in wt and in *eds1-2* mutant plants, pointing towards an *EDS1*-independancy of the regulation of these genes, also downstream from AvrRps4.

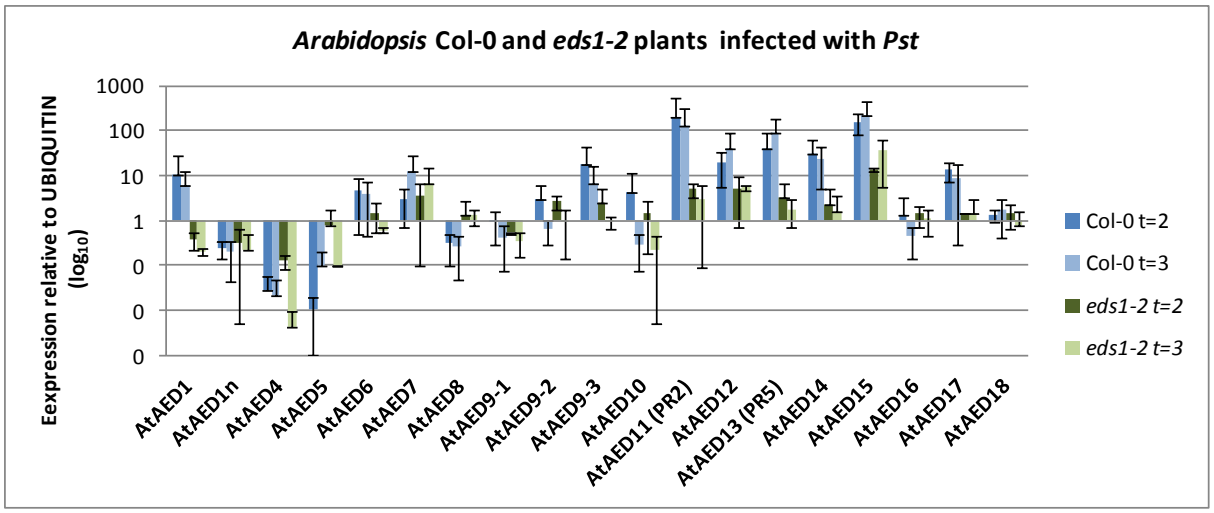


Figure 21: Arabidopsis Col-0 wild-type and *eds1-2* plants treated with *Pst*. Samples were taken two and three days after infiltration.

Experiments were repeated three times in Col-0 wild type plants with comparable results. The analysis for the *eds1-2* plants was conducted twice with comparable results.

Figure 21 shows the regulation of the expression of the different *AED* genes after infection of plants with *Pst* as compared to their expression in untreated plants. Genes are detectable that are up-regulated (e.g. *AtAED17*), down-regulated (e.g. *AtAED4* and *AtAED5*) or not regulated (e.g. *AtAED16*) in wild type plants. Again, the gene cluster from *AtAED11* to *AtAED15* shows an up-regulation, especially in the wild type *Arabidopsis* plants. *AtAED15* displays an up-regulation also in the mutant of about 10% as compared to its regulation in wt plants. *AtAED1* is up-regulated in the wild type, but not in the mutant, whereas *AtAED7* shows an up-regulation in both plant lines.

Table 23: Overview of the regulation of the different *AED* genes upon infection.

The table summarizes the expression regulation of the *AED* genes averaged over two time points in infected *Arabidopsis* wild type plants as compared to MOCK-treated plants. (Partially) *EDS1*-dependent regulations are highlighted in yellow. up=up-regulation as compared to MOCK, neutral=no regulation as compared to MOCK and down=down-regulation as compared to MOCK.

Treatment	***Pst AvrRpm1***			***Pst AvrRps4***			***Pst***		
Gene/Regulation	**up**	**neutral**	**down**	**up**	**neutral**	**down**	**up**	**neutral**	**down**
AtAED1		**X**			**X**			**X**	
AtAED1n		**X**			**X**			**X**	
AtAED4			**X**			**X**			**X**
AtAED5			**X**		**X**				**X**
AtAED6		**X**			**X**			**X**	
AtAED7		**X**				**X**		**X**	

Table 24 continued	**Treatment**	***AvrRpm1***		***AvrRps4***		***Pst***	
AtAED8			x		x		x
AtAED9-1		x			x		x
AtAED9-2		x			x	x	
AtAED9-3		x		x		x	
AtAED10			x		x		x
AtAED11 (PR2)		x		x		x	
AtAED12		x		x		x	
AtAED13 (PR5)		x		x		x	
AtAED14		x		x		x	
AtAED15			x		x		x
AtAED16			x		x		x
AtAED17			x		x	x	
AtAED18			x		x		x

Table 23 summarizes the data from Figure 18 through Figure 21. It outlines the regulation of the expression of the *AED* genes in infected wild type plants as compared to their regulation upon MOCK treatment. If genes are specifically regulated in infected wild type plants, but not or significantly less in the *eds1-2* mutant, they are considered (partially) *EDS1*-dependent. *EDS1*-dependent regulations are highlighted in yellow.

3.3 The relationship between *At*AED9-1, *At*AED9-2 and *At*AED9-3

For further physiological studies this thesis focuses on the AED9 proteins. *At*AED9-1, *At*AED9-2 and *At*AED9-3 are three closely related members of the 18 member family of legume lectin-like proteins of *Arabidopsis*. Two AED9 proteins were identified as part of the integrated proteomic approach described above. *At*AED9-1 was found in the LC-MS/MS analysis and *At*AED9-3 was found using ICPL. *AtAED9-2* was integrated in further studies of the AED9 proteins, because the similarity with *AtAED9-1* is around 89%.

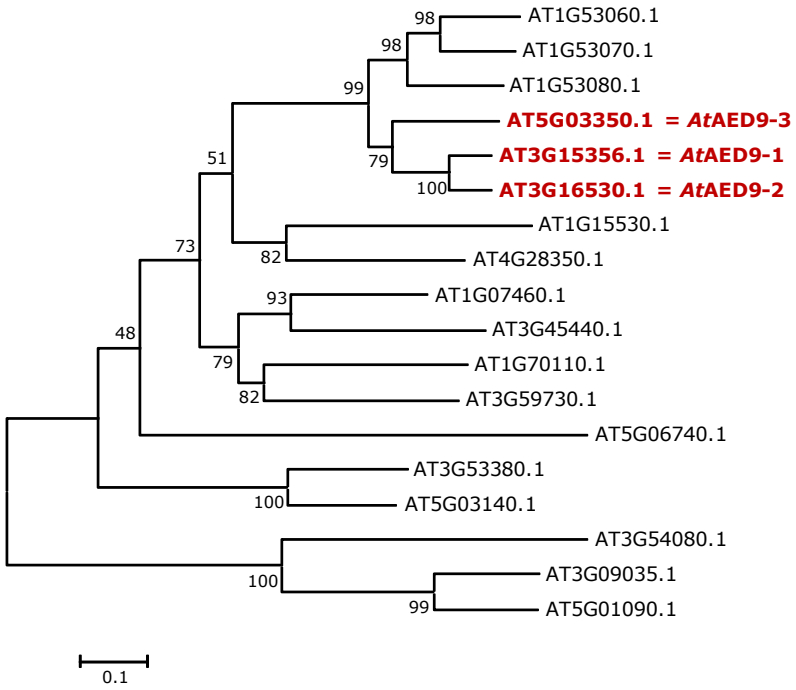


Figure 22: Phylogenetic tree based on the similarity of the coding sequences of the 18 members of the legume lectin-like protein family.

The phylogenetic tree was built using Molecular Evolutionary Genetics Analysis Software (MEGA). Candidates found in the proteomic approach are highlighted in red (*At*5G03350 = *At*AED9-3, *At*3G15356 = *At*AED9-1, *At*3G16530 = *At*AED9-2)

According to the phylogenetic tree of the *Arabidopsis* legume lectin-like genes (Figure 22), the subgroup of the three *AtAED9* genes is closely related, where *AtAED9-1* and *AtAED9-2* are more similar to each other than to *AtAED9-3*. An alignment of the amino acid sequences of the three AED9 proteins is shown in Figure 45 (supplement). Table 24 provides additional information about the predicted function and cellular location of the three *At*AED9 proteins.

Table 24: Predicted function and cellular location of *At*AED9-1, *At*AED9-2 and *At*AED9-3 obtained from *uniprot.org* and/or *arabidopsis.org*

Protein	Biological process	Cellular compartment	Molecular function
*At*AED9-1 (*At3G15356*)	defense response to fungus, incompatible interaction	apoplast, cell wall	sugar binding
*At*AED9-2 (*At3G16530*)	defense response to fungus, response to chitin	apoplast, nucleus, plant-type cell wall	sugar binding
*At*AED9-3 (*At5G03350*)	kinase, transferase	apoplast, cell wall, chloroplast	kinase activity, sugar binding

3.4 SAR experiments with *aed9-1* and *aed9-3*

3.4.1 SAR experiments with *aed9-1* and *aed9-3* using *Pst AvrRpm1* to induce SAR

To test if *At*AED9-1 and *At*AED9-3 possibly play a role in SAR, T-DNA insertion KO lines of the respective genes were tested for *P. syringae*-induced SAR. In this experiment *Pst AvrRpm1* was used to trigger SAR.

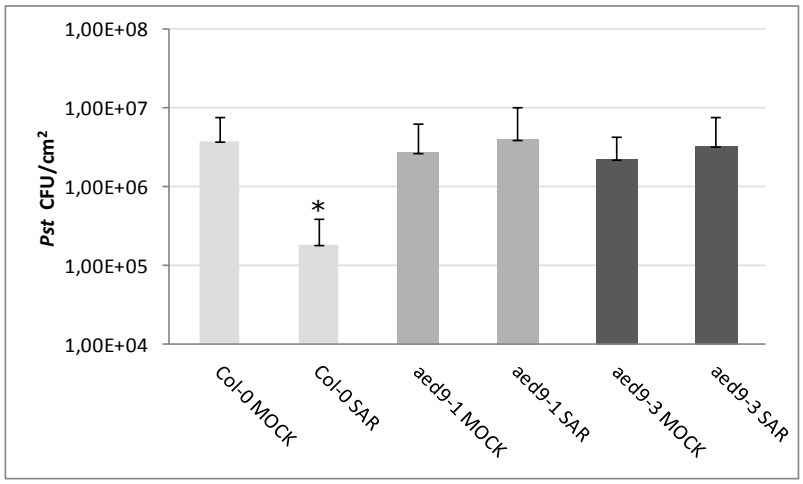


Figure 23: SAR experiment with Col-0 (light grey bars) *Arabidopsis* plants compared to *aed9-1* (grey bars) and *aed9-3* (dark grey bars) mutants.

Growth of a 2° inoculum of *Pst* was measured systemic to a 1° Mock (10 mM $MgCl_2$) or SAR-inducing (*Pst AvrRpm1*) treatment.

Asterisks directly above each set of bars indicate statistically significant differences (* $P < 0.05$, Student's t test).
Experiments were repeated five times with comparable results.

If Col-0 wild type plants were primed with *Pst AvrRpm1* in the primary infected tissue (1°), *Pst* growth was reduced upon a secondary infection of the systemic tissue (2°) (Figure 23). By contrast, 2° *Pst* growth was the same in Mock-treated as compared to SAR-induced *aed9-1* or *aed9-3* mutant plants. Thus, SAR was not detectable in the *aed9-1* and *aed9-3* mutants.

3.4.2 SAR experiments with *aed9-1* and *aed9-3* using *Pst AvrRps4* to induce SAR

Figure 24 shows a typical result of a SAR experiment using *Pst AvrRps4* as a 1° inoculum to trigger SAR.

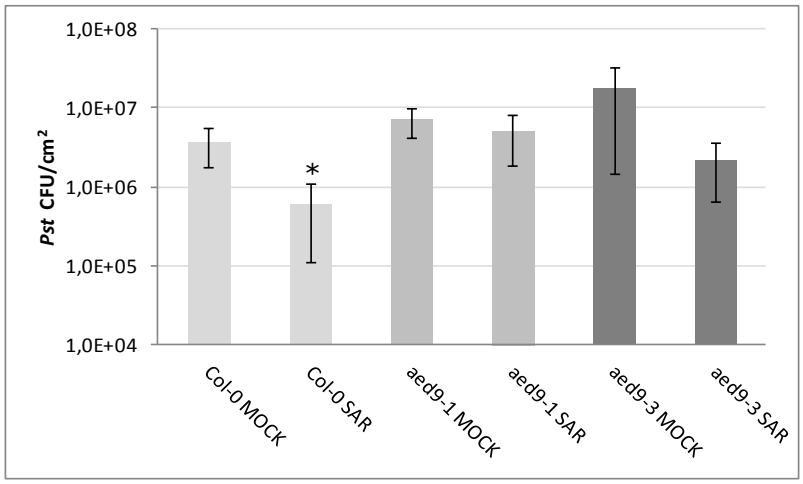


Figure 24: SAR experiment with Col-0 (light grey bars) *Arabidopsis* plants compared to *aed9-1* (grey bars) and *aed9-3* (dark grey bars) mutants.

Growth of a 2° inoculum of *Pst* was measured systemic to a 1° Mock (10 mM $MgCl_2$) or SAR-inducing (*Pst AvrRps4*) treatment.

Asterisks directly above each set of bars indicate statistically significant differences (* P < 0.05, Student's t test).
Experiments were repeated three times with comparable results.

If Col-0 wild type plants were primed with *Pst AvrRps4* in the primary infected tissue (1°), *Pst* growth was reduced upon a secondary infection of the systemic tissue (2°) (Figure 24). By contrast, 2° *Pst* growth was the same in Mock-treated as compared to SAR-induced *aed9-1* plants. Thus, SAR was not detectable in the *aed9-1* mutant. Although growth of the 2° inoculum was reduced in the systemic tissue of *aed9-3* mutant plants locally infected with *Pst AvrRps4* as compared to mock-pretreated plants, this difference was not statistically significant in four biologically independent repetitions of this experiment. Therefore, SAR was compromised in the *aed9-3* mutant as compared to wt plants.

3.5 *P. syringae* growth curves in *eds1-2*, *aed9-1* and *aed9-3*

3.5.1 Behavior of *eds1-2* in a growth curve analysis using *Pst AvrRpm1*, *Pst AvrRps4* and *Pst*

As *EDS1* is a gene that plays an important role in disease resistance and especially in SAR, the behavior of the corresponding mutant is of special interest in a growth curve experiment using *Pst AvrRpm1*.

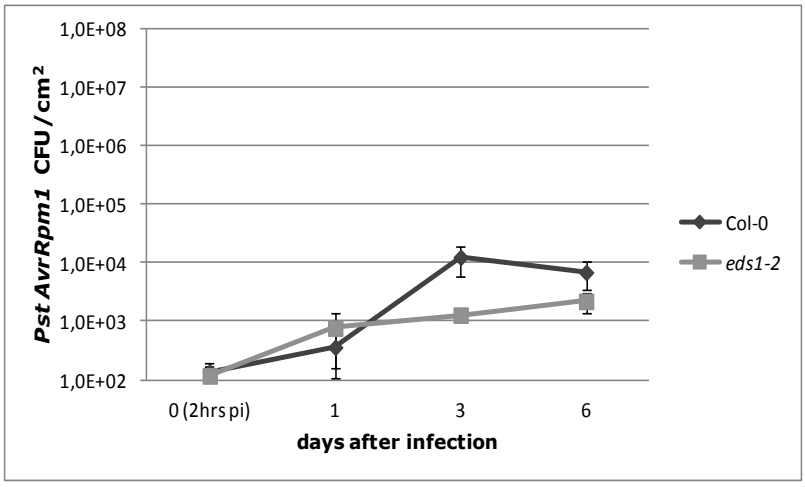


Figure 25: *Pst AvrRpm1* growth curve in Col-0 (diamond symbols) *Arabidopsis* plants as compared to *eds1-2* (square symbols) mutants.

Growth of *Pst AvrRpm1* was measured at four different time points: 0 (2hrs pi), 1, 3 and 6 days after infection. Time point 0 was used to show that all plants were infiltrated with the same concentration of bacteria.

Restricted growth was detected of *Pst AvrRpm1* in both wild type and *eds1-2* mutant plants (Figure 25). As *Pst AvrRpm1* growth was comparable in both plant lines, published data were confirmed that ETI downstream from AvrRpm1 and its cognate R protein RPM1 is not dependent on EDS1 (Aarts, Metz et al. 1998). Growth of *Pst AvrRps4* was significantly higher in *eds1-2* mutants as compared to wild type plants (Figure 26), again confirming published data that ETI downstream from AvrRps4 and its cognate R protein RPS4 is dependent on EDS1 (Aarts, Metz et al. 1998).

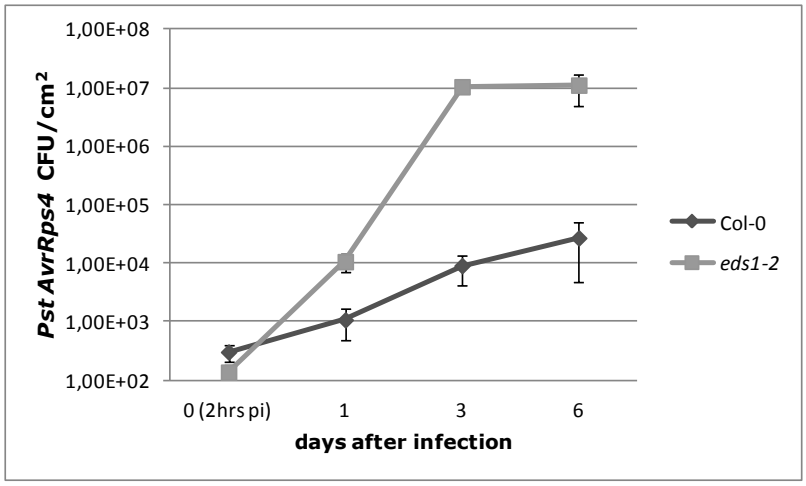


Figure 26: *Pst AvrRps4* growth curve in Col-0 (diamond symbols) *Arabidopsis* plants as compared to *eds1-2* (square symbols) mutants.

Growth of *Pst AvrRps4* was measured at four different time points: 0 (2hrs pi), 1, 3 and 6 days after infection. Time point 0 was used to show that all plants were infiltrated with the same concentration of bacteria.

3.5.2 Growth curves of *Pst*, *Pst AvrRpm1* and *Pst AvrRps4* in *aed9-1* and *aed9-3*

In order to characterize the behavior of the two KO mutant lines *aed9-1* and *aed9-3* when inoculated with *Pst* carrying different effectors, growth curve analyzes of three different bacterial strains were performed. As shown above, *Pst AvrRpm1* induces an *EDS1*-independent defense response, whereas *Pst AvrRps4* induces an *EDS1*-dependent response. *Pst* is a virulent strain inducing *EDS1*-dependent basal defenses.

The *aed9-1* and *aed9-3* mutants were infiltrated with *Pst*, titers of which were measured from 0 (2hrs pi) to 6 days after infection (Figure 27).

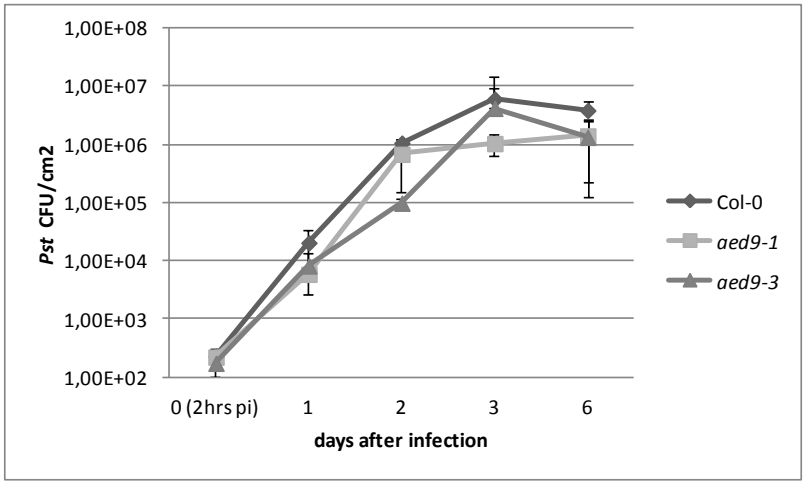


Figure 27: *Pst* growth curve in Col-0 (diamond symbols) *Arabidopsis* plants as compared to *aed9-1* (square symbols) and *aed9-3* (triangular symbols) mutants.

Growth of *Pst* was measured at four different time points: 0 (2hrs pi), 1, 3 and 6 days after infection. Time point 0 was used to show that all plants were infiltrated with the same concentration of bacteria.

Col-0 wild type plants support a steady growth of *Pst* from day 1 until day 3 after infection with no further increase until day 6. *Pst* growth was essentially the same in wild type and both *aed9-1* and *aed9-3* mutant plants, indicating that all genotypes behave similarly when infected with *Pst*. Thus, basal resistance does not appear to be affected by *AED9-1* or *AED9-3.*

Subsequently, the behavior of *aed9-1* and *aed9-3* was evaluated in a growth curve experiment using *Pst AvrRpm1*, titers of which were measured from 0 (2hrs pi) to 6 days after infection (Figure 28).

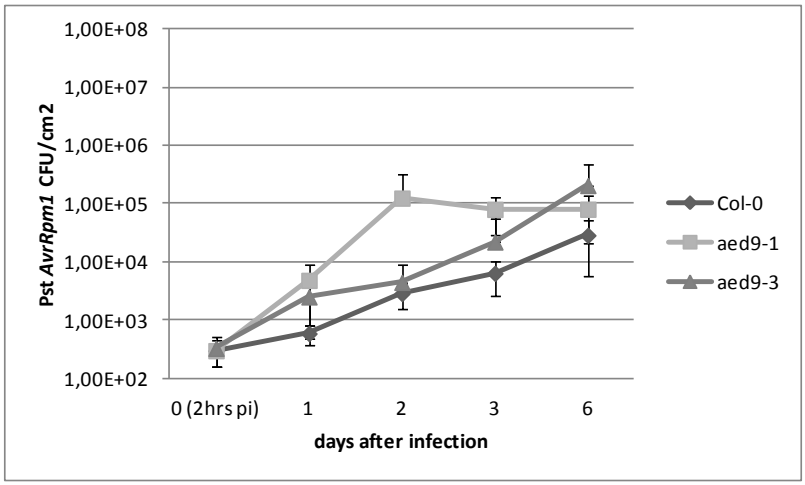


Figure 28: *Pst AvrRpm1* growth curve in Col-0 (diamond symbols) *Arabidopsis* plants as compared to *aed9-1* (square symbols) and *aed9-3* (triangular symbols) mutants (preliminary data).

Growth of *Pst AvrRpm1* was measured at four different time points: 0 (2hrs pi), 1, 3 and 6 days after infection. Time point 0 was used to show that all plants were infiltrated with the same concentration of bacteria.

Col-0 supports a steady growth of *Pst AvrRpm1* from day 1 until day 6 after infection. *Pst AvrRpm1* growth was similar in both mutants as compared to wild type plants. Any growth differences between genotypes recorded in the experiment shown in Figure 28 were not reproducible. Thus, both *aed9-1* and *aed9-3* behave similarly as *eds1-2* in interaction with *Pst AvrRpm1.*

Finally, the same plant lines were tested in a growth curve experiment after infection with *Pst AvrRps4*, titers of which were measured from 0 (2hrs pi) to 6 days after infection (Figure 29).

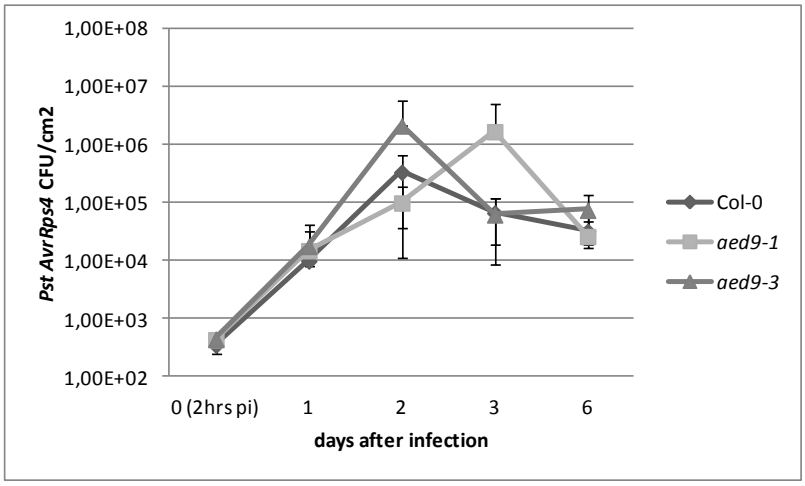


Figure 29: *Pst AvrRps4* growth curve in Col-0 (diamond symbols) *Arabidopsis* plants as compared to *aed9-1* (square symbols) and *aed9-3* (triangular symbols) mutants (preliminary data).

Growth of *Pst AvrRps4* was measured at four different time points: 0 (2hrs pi), 1, 3 and 6 days after infection. Time point 0 was used to show that all plants were infiltrated with the same concentration of bacteria.

Col-0 supports a steady growth of *Pst AvrRps4* from day 1 until day 2 after infection. Subsequently, bacterial growth is in a steady state until at least 6 days after infection. The same applies for *aed9-3* with no significant difference in *Pst AvrRps4* growth as compared to wild type plants. In the experiment shown in Figure 28, *Pst AvrRps4* growth appears somewhat delayed in *aed9-1* reaching its peak on day 3 after infection. Since this result was not reproducible in other experiments and since the highest titer of *Pst AvrRps4* measured in *aed9-1* was similar to that in wild type and *aed9-3* plants, the data indicate that *EDS1*-dependent ETI downstream from AvrRps4 is not affected in either *aed9* mutant.

3.6 Expression analysis of the three lectin genes

In this part of the study, the expression of *AtAED9-1*, *AtAED9-2* and *AtAED9-3* was examined during SAR induced by either *Pst AvrRpm1* or *Pst AvrRps4*. Samples were derived from SAR experiments similar to the ones shown in Figure 23 and Figure 24.

3.6.1 qRT-PCR analysis of *AtAED9-1*, *AtAED9-2* and *AtAED9-3* during a SAR-experiment, where SAR was induced with *Pst AvrRpm1*

The results are presented in two figures; one is showing the gene expression in the local, infected (1°) tissue (Figure 30), whereas the other one illustrates the gene expression in the systemic tissue (2°) (Figure 31). Furthermore, the expression of *AtAED9-2* and *AtAED9-3* in the *aed9-1* knock out is shown (Figure 32).

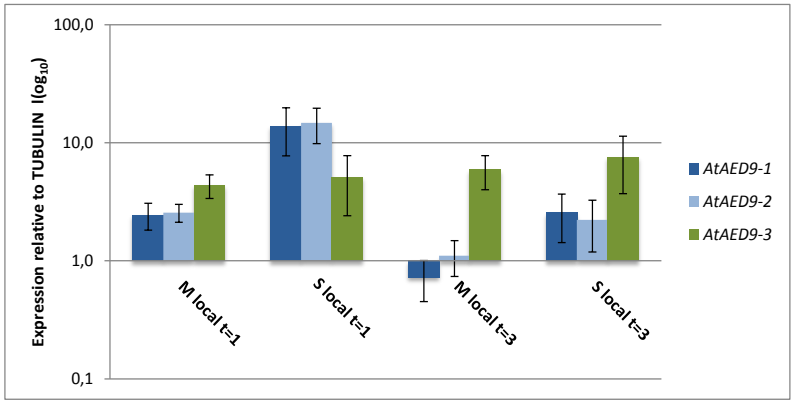


Figure 30: Expression of *AtAED9-1* (dark blue), *AtAED9-2* (light blue) and *AtAED9-3* (green) relative to *TUBULIN* in the local, infected tissue (1°) of *Arabidopsis* wild type plants one day (t=1) and three days (t=3) after infection with *Pst AvrRpm1*.

M = MOCK-treated tissue; S = *Pst AvrRpm1*-treated tissue.
Experiments were repeated two times using two housekeeping genes in two biological replicates with comparable results.

The expression of *AtAED9-1* and *AtAED9-2* is up-regulated in wild type *Arabidopsis* plants one day after infection with *Pst AvrRpm1* (S local t=1) showing about a 10-fold induction compared to untreated plants and a roughly three-fold induction compared to mock-treated plants. This expression goes down at three days after infection (S local t=3). MOCK-treated plants, infiltrated with 10mM $MgCl_2$ solution, are showing a gene induction one day after treatment (M local t=1), but not after three days (M local t=3). Taken together, both *AtAED9-1* and *AtAED9-2* are induced by *Pst AvrRpm1* treatment one day after infection in the local tissue (1°) as compared to MOCK or untreated plants. By contrast, expression of *AtAED9-3* is induced to a similar level by MOCK treatment or infection at both time points. Therefore, *AtAED9-3* is not up-regulated by the infection with *Pst AvrRpm1* in the local tissue (1°).

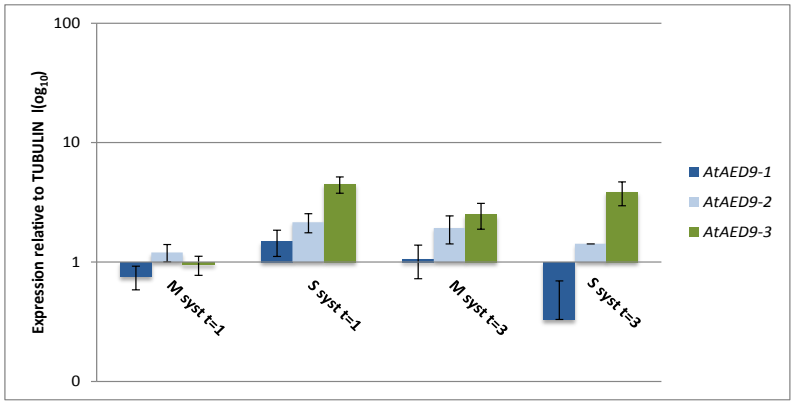


Figure 31: Expression of *AtAED9-1* (dark blue), *AtAED9-2* (light blue) and *AtAED9-3* (green) in the systemic tissue (2°) of *Arabidopsis* wild type plants one day (t=1) and three days (t=3) after infection with *Pst* AvrRpm1.

M = MOCK-treated tissue; S = tissue systemic to *Pst AvrRpm1*-treated tissue.
Experiments were repeated two times using two housekeeping genes in two biological replicates with comparable results.

Subsequently, expression of the *AtAED9* genes was evaluated in the systemic tissue (2°) one and three days after a local *Pst AvrRpm1* infection (Figure 31). *AtAED9-1* and *AtAED9-2* expression was not induced in the systemic (2°) tissue at one or three days after the treatment of the local tissue (1°) with MOCK solution or *Pst AvrRpm1*. However, one day after treatment the expression of *AtAED9-3* was enhanced especially in the systemic tissue (2°) of locally (1°) *Pst AvrRpm1*-treated plants.

Due to its high similarity with *AtAED9-1* and to test if *AtAED9-2* could potentially be functionally redundant with *AtAED9-1* the expression of *AtAED9-2* and *AtAED9-3* was evaluated in the *aed9-1* KO Arabidopsis plants (Figure 32).

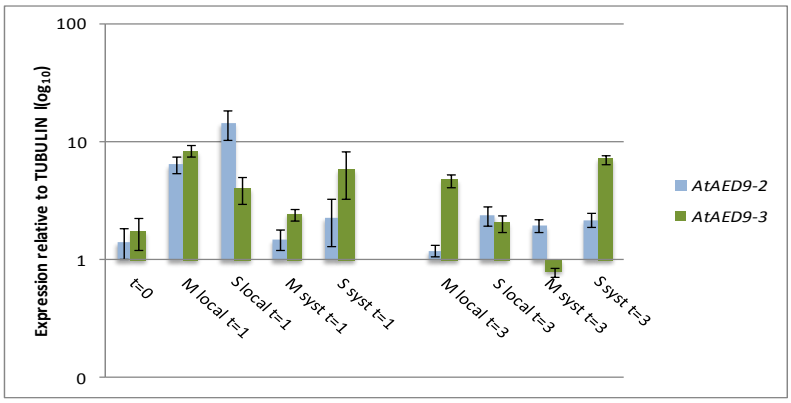


Figure 32: Expression of *AtAED9-2* (light blue) and *AtAED9-3* (green) in *Arabidopsis aed9-1* KO plants one day (t=1) and three days (t=3) after infection with *Pst* AvrRpm1

M = MOCK-treated plants; S = *Pst AvrRpm1*-treated plants.
Experiments were repeated two times using two housekeeping genes in two biological replicates with comparable results.

The expression of *AtAED9-1* in *aed9-1* is clearly down-regulated/not detectable by qRT-PCR, showing that *AtAED9-1* is stably knocked out (Figure 46, supplement). Moreover, RT-PCR (reverse transcriptase) using primer HB-P11 and HB-P12 (Table 26) proved that an *AtAED9-1* transcript is not detectable in the *aed9-1* KO mutant (Figure 48, supplement).

AtAED9-2 expression is up-regulated in the local tissue (1°) one day after treatment of *aed9-1* with either MOCK solution or *Pst AvrRpm1*. There is no up-regulation detectable in the remaining tissues. *AtAED9-3* shows an up-regulation in nearly all the tissues, but worth mentioning is the up-regulation in the systemic tissue (2°) one and three days after infecting the local (1°) tissue with *Pst AvrRpm1*.

3.6.2 qRT-PCR analysis of *AtAED9-1*, *AtAED9-2* and *AtAED9-3* during a SAR-experiment, where SAR was induced with *Pst AvrRps4*

In this paragraph, the expression regulation of *AtAED9-1*, *AtAED9-2* and *AtAED9-3* is shown during a SAR experiment, in which SAR was induced by *Pst AvrRps4*. The gene expression in the local tissue (1°) is shown in Figure 33.

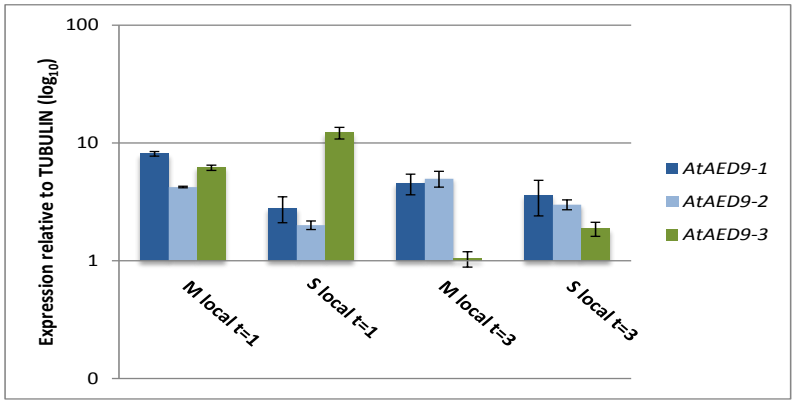


Figure 33: Expression of *AtAED9-1* (dark blue), *AtAED9-2* (light blue) and *AtAED9-3* (green) in the local tissue (1°) of *Arabidopsis* wild type plants one day (t=1) and three days (t=3) after infection with *Pst AvrRps4*

M = MOCK-treated tissue; S = *Pst AvrRps4*-treated tissue.
Experiments were repeated two times using two housekeeping genes in two biological replicates with comparable results.

AtAED9-1 and *AtAED9-2* expression is not induced after infection with *Pst AvrRps4* compared to the MOCK-treated *Arabidopsis* plants. *AtAED9-3* is induced after infection with *Pst* AvrRps4 one day after treatment (S local t=1). This expression is going down at day three after infection. Figure 34 illustrates the gene expression behavior of the *AED9* genes in the systemic (2°) tissue (Figure 34).

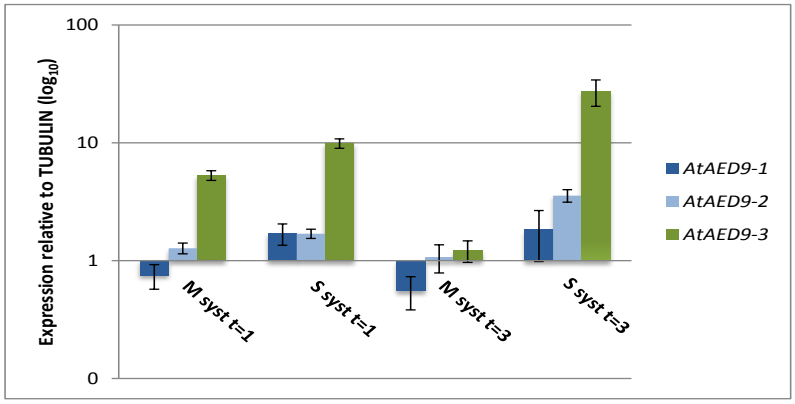


Figure 34: Expression of *AtAED9-1* (dark blue), *AtAED9-2* (light blue) and *AtAED9-3* (green) in the systemic tissue (2°) of *Arabidopsis* wild type plants one day (t=1) and three days (t=3) after infection with *Pst* AvrRps4

M = MOCK-treated tissue; S = tissue systemic to *Pst AvrRps4*-treated tissue.
Experiments were repeated two times using two housekeeping genes in two biological replicates with comparable results.

On the one hand, the expression pattern of *AtAED9-1* and *AtAED9-2* is similar in all the different tissues tested, indicating that these two genes are not induced in the systemic tissue (2°) of infected as compared to MOCK pretreated plants. On the other hand, *AtAED9-3* is induced in the systemic tissue (2°) one day and especially three days after infection of the local tissue with *Pst AvrRps4*.

Comparable to the experiment, in which the *Arabidopsis* plants were treated with *Pst AvrRpm1*, the behavior of *AtAED9-2* and *AtAED9-3* was evaluated in a SAR experiment in the *aed9-1* KO mutant with *Pst AvrRps4* (Figure 35).

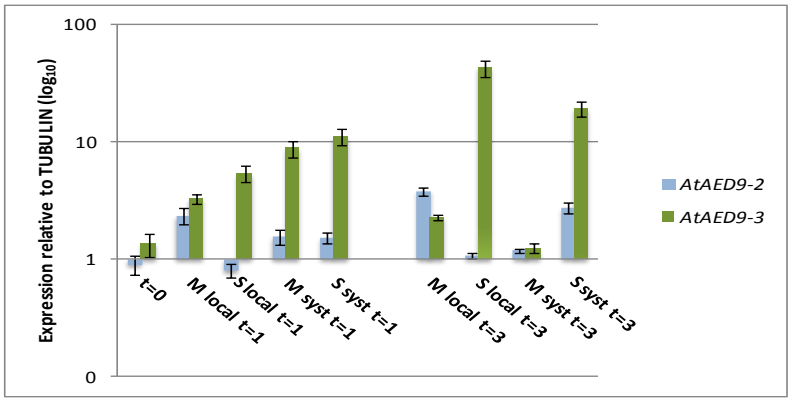


Figure 35: Expression of *AtAED9-2* (light blue) and *AtAED9-3* (green) in *Arabidopsis aed9-1* KO plants one day (t=1) and three days (t=3) after infection with *Pst* AvrRps4

M = MOCK-treated plants; S = *Pst AvrRps4*-treated plants.
Experiments were repeated two times using two housekeeping genes in two biological replicates with comparable results.

AtAED9-2 expression is up-regulated in the local tissue (1°) one and three days after treatment with MOCK solution. A comparable induction of *AtAED9-2* was detectable in the systemic tissue (2°) three days after local treatment with *Pst AvrRps4*. *AtAED9-3* shows an up-regulation in nearly all the tissues, especially in the local (1°) and systemic tissues (2°) three days after infecting the local (1°) tissue with *Pst AvrRps4*.

3.7 *At*AEDs in tobacco – establishment of a new, medium-high throughput, *in planta* screen for SAR signaling proteins

3.7.1 Systemic TMV assay with *At*AED1, *At*AED4, and *At*AED5 in *Nicotiana tabacum*

Systemic TMV assays were established to clarify a potential role in SAR of three AEDs. Therefore, *Nicotiana tabacum* (tobacco) Xanthi nc containing the TMV resistance gene *N* (Park, Kaimoyo et al. 2007) and a Xanthi nc *NahG* transgenic line (Gaffney, Friedrich et al. 1993) were used. *At*AED1 (aspartic protease family protein), *At*AED4 (GDSL-motif lipase/hydroxylase family protein), *At*AED5 (GDSL-motif lipase/hydroxylase family protein), or a mixture of *At*AED4 and *At*AED5 (1:1) were expressed in three leaves of six-week-old tobacco plants by using *Agrobacterium tumefaciens*-mediated transient expression (agroinfiltration). Agroinfiltration was applied to the central third of each treated leaf (Figure 36) on what is referred to here as Day 0.

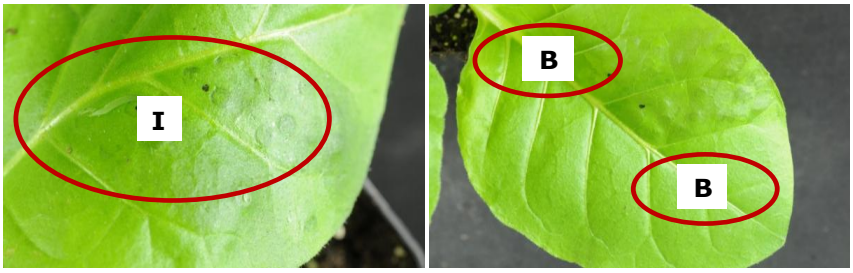


Figure 36: Analyzed parts of an agroinfiltrated tobacco leaf.

Picture on the left shows an infiltrated site on a tobacco leaf (I=infiltrated region).
Picture on the right shows un-infiltrated or bordering parts of an infiltrated site of a tobacco leaf (B=border region).

Because the infiltrated constructs carry the *At*AED coding sequences downstream from estradiol-inducible promoters, the agroinfiltrated leaves were sprayed with 30µM of estradiol one day after agroinfiltration (Day 1). On Day 3, samples were taken from the infiltrated and bordering regions of the agroinfiltrated leaves. Protein expression was analysed by Western blot using antibodies against the HA-tag that was attached to the C-terminus of each *At*AED. As controls, plants were infiltrated with buffer (MMA) or with 'empty' (non-vector-containing) *A. tumefaciens* (GV3101; same strain as used for agroinfiltration of the *At*AEDs). A representative western blot analysis of infiltrated (I) and bordering region (B) samples is shown in Figure 37.

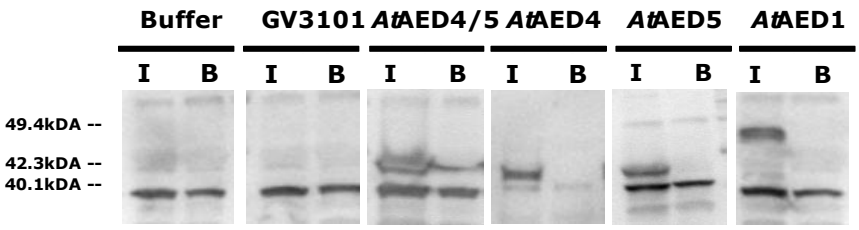


Figure 37: Western blot analysis of local tissue (1°) tissue in order to evaluate the transient over-expression of proteins-of-interest.

I=infiltrated region; B=un-infiltrated or border region

After the infiltration of buffer or GV3101 into the local tissue (1°), only a non-specific background protein band could be detected both in the infiltrated (I) and in the un-infiltrated region (B). The expression of *At*AED4/*At*AED5 (1:1 mixture), *At*AED4, *At*AED5, and *At*AED1 was verifiable in the infiltrated region (I), but not in the un-infiltrated region (B) of agroinfiltrated leaves. In the experiment shown in Figure 36 co-expressed *At*AED4 and *At*AED5 were detected also in the un-infiltrated region (B) of the agroinfiltrated leaf. In this case, a weaker protein signal was detected in the un-infiltrated part (B) as compared to the infiltrated region (I), which leads to the assumption that *At*AED4 and *At*AED5 together might be mobile. However, this observation was made once and could not be repeated.

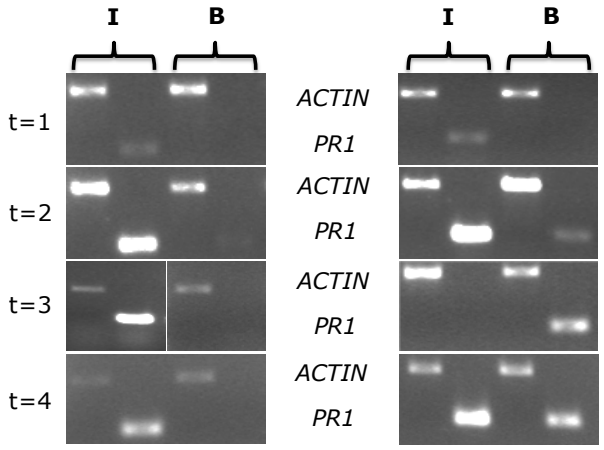


Figure 38: RT-PCR of *PR1* in infiltrated (I) and bordering tissue (B) at one until four days after transient over expression of *At*AED1 (left side) and GUS (right side), respectively.

RT-PCR of *ACTIN* was used as equal loading control.

Figure 38 shows the expression of *PR1* in infiltrated and bordering tissue of agroinfiltrated tobacco leaves after transient over expression of *At*AED1 and GUS (β-

glucuronidase – an enzyme that catalyses the hydrolysis of a wide variety of glucuronides (Jefferson 1989)), respectively. In this experiment, expression of GUS was included as a negative (protein) control. *PR1* was expressed in the infiltrated tissue (I) from one day until four days after transient over expression of *At*AED1, whereas no *PR1* expression was detectable in the bordering tissue (B) (Figure 38, panels on left). Transient expression of the control protein GUS induced expression of *PR1* in the infiltrated tissue (I), but also in the bordering tissue (B) from two until four days after agroinfiltration (, panels on right). Since agroinfiltration of the control protein induced the SAR marker gene *PR1* in the bordering tissue, some distal signaling appears to be triggered by *A. tumefaciens.* Such signaling is reproducibly inhibited by *At*AED1, confirming a possible feedback inhibitory function of *At*AED1 during SAR establishment in *Arabidopsis* (Wenig, Knappe, Parker, and Vlot, unpublished).

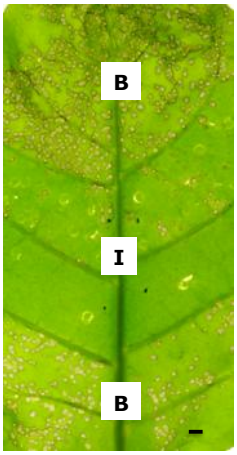


Figure 39: Local (1°)/Agroinfiltrated tobacco leaf infected with TMV subsequent to the infiltration of GV3101 in one third (I) of the leaf.

I represents GV3101-infiltrated region; B represents the bordering part. The whole leaf was infected with TMV 7 days after agroinfiltration of I.

bar=1cm

To assess the resistance-inducing capacity of the *At*AEDs, studies were carried out, in which the local/agroinfiltrated tissue (1°) of tobacco plants was infected with TMV. Local TMV infections were done subsequent to GV3101 or buffer infiltration and subsequent to transient over expression of *At*AED1, *At*AED4 and *At*AED5. All agroinfiltrated leaves responded the same, irrespective of whether GV3101 was used with or without *AtAED*-encoding construct (Figure 39). Upon TMV infection, the agroinfiltrated region (I) stayed free of TMV lesions, whereas no differences in TMV lesion size or number could be detected on the tissue bordering (B) the different pretreatments. Therefore, already GV3101 itself induces a local defense reaction. This result correlates well with a previously carried out study, in which *A. tumefaciens* triggered exactly this defense reaction, whereas heat-killed *A. tumefaciens* did not (Pruss, Nester et al. 2008).

Subsequently, the systemic resistance-inducing capacity of the *At*AEDs was tested. To this end, three leaves systemic to the agroinfiltrated leaves were infected with TMV on Day 7. In this experiment the effect on systemic resistance to TMV was evaluated of

transiently expressed *At*AED1, *At*AED4, *At*AED5 and the *At*AED4/*At*AED5 (1:1) mixture. Figure 40 shows TMV lesions seven days after infection of the systemic, defense-induced leaves (2°) distal to the different localized (1°) treatments of wild type tobacco Xanthi nc (Figure 40).

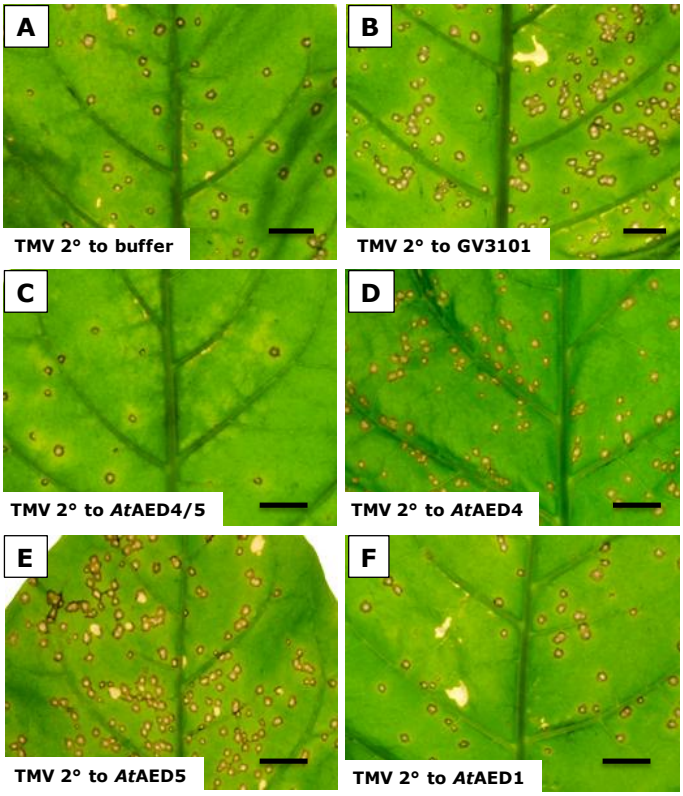


Figure 40: TMV infection of systemic, defense-induced leaves (2°) of *Nicotiana tabacum* (tobacco) Xanthi nc containing the TMV resistance gene *N*

A) Leaf systemic (2°) to localized (1°) buffer treatment

B) Leaf systemic (2°) to localized (1°) GV3101 treatment

C) Leaf systemic (2°) to localized (1°) *At*AED4/5 (1:1) treatment

D) Leaf systemic (2°) to localized (1°) *At*AED4 treatment

E) Leaf systemic (2°) to localized (1°) *At*AED5 treatment

F) Leaf systemic (2°) to localized (1°) *At*AED1 treatment

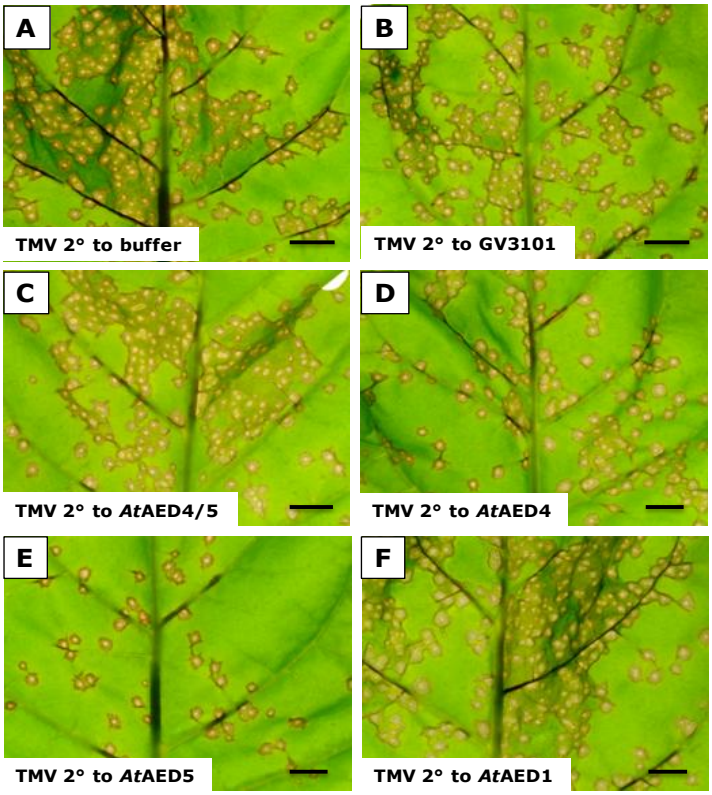
bars=1cm

The defense-inducing potential of the different *At*AED proteins was judged in comparison to GV3101 (Figure 40B). 2° TMV lesions on plants (locally) pre-treated with GV3101 grew somewhat faster than on plants pre-treated with buffer solution, indicating that *A. tumefaciens* itself does not trigger SAR in these tobacco experiments. From the remaining pictures (Figure 40C-F) the defense-inducing potential of *At*AED4/*At*AED5 (1:1 mixture) (C), *At*AED4 (D), *At*AED5 (E), and *At*AED1 (F) can be deduced.

Plants pre-treated with *At*AED4/*At*AED5 (1:1 mixture) support relatively few and small TMV lesions on the systemic tissue. Therefore, *At*AED4 and *At*AED5 together trigger enhanced systemic resistance or SAR. The *At*AED4/*At*AED5 mixture induced this effect in all examined plants. Hence, both proteins together have a SAR-inducing potential as compared to GV3101. By contrast, *At*AED4 and *At*AED5 alone do not seem to enhance the resistance against TMV in the systemic tissue in the experiment shown in Figure 40. Over the course of multiple repetitions *At*AED4 and *At*AED5 each individually induced systemic resistance against TMV in approximately 50% of the examined plants. Pre-treatment of tobacco plants with *At*AED1 mostly did not show a SAR-inducing protential of this protein, which was therefore concluded not to trigger SAR in tobacco.

SAR normally is dependent on SA, especially in the systemic tissue. To test if the systemic resistance triggered by *At*AED4, *At*AED5, and/or *At*AED4/*At*AED5 also is dependent on SA, the systemic TMV assay was repeated in *NahG* transgenic tobacco Xanthi nc. Figure 41 shows TMV lesions seven days after infection of the systemic, defense-induced leaves (2°) distal to the different localized (1°) treatments of the *Nicotiana tabacum* (tobacco) Xanthi nc *NahG* transgenic line (Gaffney, Friedrich et al. 1993).

Figure 41: TMV lesions on systemic, defense-induced leaves (2°) of *Nicotiana tabacum* (tobacco) Xanthi nc *NahG*



G) Leaf systemic (2°) to localized (1°) buffer treatment

H) Leaf systemic (2°) to localized (1°) GV3101 treatment

I) Leaf systemic (2°) to localized (1°) *At*AED4/5 (1:1) treatment

J) Leaf systemic (2°) to localized (1°) *At*AED4 treatment

K) Leaf systemic (2°) to localized (1°) *At*AED5 treatment

L) Leaf systemic (2°) to localized (1°) *At*AED1 treatment

bars=1cm

2° TMV lesions on plants (locally) pre-treated with GV3101 grew similar as on plants pre-treated with buffer solution (Figure 41A and B).

The defense-inducing potential of the different *At*AED proteins was judged in comparison to GV3101 (Figure 41B). From the remaining pictures (Figure 41C-F) the defense-inducing potential of *At*AED4/*At*AED5 (1:1 mixture) (C), *At*AED4 (D), *At*AED5 (E), and *At*AED1 (F) can be deduced.

Plants pre-treated with the proteins-of-interest did not show enhanced resistance to TMV in the systemic tissue (2°). Hence, no protein or protein pair has a SAR inducing potential in *NahG* transgenic plants. Thus, the *At*AED-induced systemic resistance in wild type tobacco is SA-dependent.

The influence of the transiently expressed *At*AED proteins on SAR induction is summarized in Figure 42.

	Buffer	GV3101	*At*AED1	*At*AED4	*At*AED5	*At*AED4/5
***N. tabacum* Xanthi nc N**	No	No	Inhibits SAR-induction	Yes (50%)	Yes (50%)	Enhances SAR-induction
N. tabacum* Xanthi nc *nahG	No	No	No	No	No	No

SAR-inducing potential

Figure 42: SAR-inducing potential of transiently expressed proteins (1°) in tobacco – overview

*At*AED4 and *At*AED5 show a SAR-inducing potential in *Nicotiana tabacum* (tobacco) Xanthi nc containing the TMV resistance gene *N*, whereas *At*AED1 even displays a SAR-inhibiting function (highlighted in red); the 1:1 mixture of *At*AED4/5 shows a strong SAR-inducing potential (highlighted in red) in all cases tested. No SAR-inducing potential was detectable in *Nicotiana tabacum* (tobacco) Xanthi nc *NahG* transgenic line.
No=no SAR-induction; Yes=SAR-induction in about 50% of the cases; On average 10 to 12 plants/repetitions were tested in *Nicotiana tabacum* (tobacco) Xanthi nc containing the TMV resistance gene *N* and on average 5 plants/repetitions were conducted using *Nicotiana tabacum* (tobacco) Xanthi nc *NahG* transgenic line.

If *Nicotiana tabacum* (tobacco) Xanthi nc containing the TMV resistance gene *N* was used buffer and GV3101 treated plants did not display SAR. As a positive control, wild type tobacco plants were infected with TMV locally (1°) and subsequently challenged with TMV systemically (pictures not shown). This resulted in significantly fewer and smaller 2° lesions and therefore a clear SAR-induction, which was, according to the lesion appearance, somewhat stronger than SAR induced by the 1:1 mixture of *At*AED4 and *At*AED5.

*At*AED4 and *At*AED5 each had SAR-inducing potential, but only in around 50% of the plants tested. An inhibitory function displayed *At*AED1, which was shown by the suppression of *PR1* in the bordering tissue (B) after transient over expression of *At*AED1 (Figure 38). By contrast, the 1:1 mixture of *At*AED4 and *At*AED5 induced SAR-like systemic resistance in 100% of the cases tested. The same treatments were applied to *Nicotiana tabacum* (tobacco) Xanthi nc *NahG* transgenic line (Gaffney, Friedrich et al. 1993). Here, no treatment induced SAR, indicating a SA-dependency of *At*AED4, *At*AED5 and the 1:1 mixture of *At*AED4/5.

Chapter 4: DISCUSSION

Intensive research on systemic acquired resistance (SAR) and especially on the elucidation of new SAR signals or potentially SAR-involved signaling proteins/components brought a lot of new insights in the recent years. Several candidate long distance signals have been proposed, naming methyl-salicylate (MeSA) as one of the most prominent ones. What is constant in SAR-research is that the "readout" of SAR is always similar: the systemic tissue (2°) shows an enhanced resistance against a broad range of pathogens after previous infection/induction of the local tissue (1°) through pathogens.

In this study we aimed to identify new "players" in the SAR signaling process by comparing the apoplastic proteomic profile of wild type and *eds1-2* mutant plants after the expression of the bacterial effector AvrRpm1 from a DEX-inducible transgene. By applying 2D-gel (Vlot *et al*., conducted at the Max Planck Institute for Plant Breeding Research, Cologne, Germany), LC-MS/MS and ICPL we identified 21 proteins that reproducibly accumulate in the apoplast of wild type plants in an *EDS1*-dependent manner. Among these 21 proteins, three proteins belong to the legume lectin-like protein family and are characterized in this study in more detail, e.g by SAR experiments with the respective KO mutants of two of the three legume lectin-like genes. The decision to focus on the three legume lectin-like genes for functional characterization was based on the fact that two out of the three corresponding proteins were found with two different proteomic methods. *At*AED9-1 was detected by LC-MS/MS and *At*AED9-3 by ICPL analysis. Moreover, both genes as well as the highly similar *AtAED9-2* co-segregate forming one branch of the phylogenetic tree of legume lectin-like genes having a very similar coding sequence (Figure 22) as well as protein sequence (Figure 45). According to literature *At*AED9-1 is involved in disease resistance (Lyou, Park et al. 2009) and is even considered to be a PR protein (Delessert, Kazan et al. 2005).

4.1 Overall outcome of the integrated proteomic approach: comparison of the different methods applied

For the integrated proteomic approach three different methods were applied: 2D-gel (Vlot *et al*., conducted at the Max Planck Institute for Plant Breeding Research, Cologne, Germany), LC-MS/MS and ICPL analysis. For all the different proteomic approaches used in this study the composition of the apoplast extraction buffer was adjusted to the analytical method. The appropriate sample preparations turned out to be the bottleneck compared to the analytical approaches themselves.

2D-PAGE analysis coupled with MS for protein identification was widely used in the last decade, also in plant-pathogen interaction studies (Perez-Bueno, Rahoutei et al. 2004) (Jones, Thomas et al. 2006) (Subramanian, Cho et al. 2009). At this point one example is pointed out to show the similarity in the number of identified proteins compared to our analysis. 2D-gel analysis was used for example to identify defense-related proteins in rice challenged with the fungal pathogen *Rhizoctonia solani* (Lee, Bricker et al. 2006). In this study the authors compared two treatments, infected vs. non-infected, and two different rice strains, resistant vs. susceptible. Out of around 1000 protein spots (Lee, Bricker et al. 2006) were able to narrow it down to 6 proteins, which accumulated in the resistant rice strain after infection as compared to the susceptible strain. These numbers are also represented in our study, where we identified 7 proteins out of around 1000 estimated proteins in the apoplast/cell wall of *Arabidopsis*.

In order to get a deeper insight into the proteome present in the apoplast of our samples, LC-MS/MS analysis was conducted. Therefore, it was indispensable to 'clean' the samples before injecting them into the HPLC in order to avoid clogging of the column. Two SPE methods were tested, from which the method purifying trypsin-digested peptides on a C18 column resulted in the most optimal protein recovery and was thus chosen as most appropriate. Without fractionation based on a 1D-gel we identified a total number of around 350 proteins per apoplast extract. Fractionation of the samples into 5 slices of a 1D-gel raised the number of identified proteins per extract to around 1150 proteins. As a result of the combined LC-MS/MS analyses, three additional AED proteins were added to the already discovered 7 originating from the 2D-gel approach.

Although methods of proteomics, especially the MS-based analytical methods, are improving, it is very hard to cover the whole proteome of a given organism or sample (Frohlich, Gaupels et al. 2012). The reasons are: each gene is not only resulting in one protein, but is able to produce a lot of different proteins, which can additionally undergo various posttranslational modifications. Moreover, there is a huge range of protein concentrations within a given sample (Corthals, Wasinger et al. 2000). Therefore, the samples can include some proteins that are high abundant, thus dominating the low abundant ones. By using 1D gel slices, we confirmed prior knowledge that fractionation of (apoplast) samples prior to LC-MS/MS results in a much higher protein identification rate due to the reduction of the complexitiy of the samples.

As the 2D-gel and LC-MS/MS analyses were aiming at a qualitative or at least semi-quantitative result, ICPL was conducted to supplement an integrative view on our samples by adding a quantitative method. In general, stable isotopes can be incorporated at different stages of a quantitative proteomics workflow: on the metabolic, protein and

peptide level. The ICPL method is based on isotopic labeling of all free amino acid groups in proteins. It has the advantages that it is applicable to any sample and that it is fast. A potential weaknes lies in the incorporation of stable isotopes at the protein level. Practically, only Lys-containing peptides and the protein N-terminus can be used for quantitation.

In our study we reduced the protein complexity of the isotope-labeled samples by fractionating the proteins on a 1D-gel prior to trypsin digestion and injection into the Orbitrap (Hu, Noll et al. 2005). In total, 759 protein IDs were detected, from which 610 were labeled and therefore quantitatively comparable. This means ~80% of the identified proteins were labeled. Similar values were published when the ICPL and iTRAQ labeling techniques (Nogueira, Palmisano et al. 2012) were compared.

Protein quantification by ICPL is measured as the heavy/light (isotope) ratio of a given peptide/protein. Spiked control proteins provide internal controls to evaluate the reliability of the heavy/light ratios measured. In this study, the outcome of the measurements of the control proteins was in accordance with the theoretically expected values. Therefore, the ICPL analyses were valid. Furthermore, PR2 and PR5 were among the statistically regulated proteins that accumulate in the apoplast of *AvrRpm1*-expressing plants in an *EDS1*-dependent manner. Because both proteins are SAR marker proteins/genes (Thomma, Penninckx et al. 2001) (Ryals, Neuenschwander et al. 1996), these findings validate the induction of the *AvrRpm1* transgene and/or SAR in our experiments. PR2 also was detectable as differentially accumulating in wild type and mutant in three independent runs of the LC-MS/MS analyses, while both PR2 and PR5 were found as part of the 2D gel approach.

By comparing the outcome of the different proteomic approaches applied in this study, proteins are mentioned here that were detected by more than one analytical approach as differentially accumulating between wild type and mutant. It emerged that *At*AED4 and *At*AED7 originating from the 2D-gel could also be detected by ICPL. Both proteins were up-regulated in the Col-0 wild type samples as compared to *eds1-2* showing a heavy/light ratio of 1.44 and 1.14 (both not statistically significant), respectively. *At*AED4 was also detectable in two repetitions of the LC-MS/MS analysis. Two proteins, which according to ICPL data were significantly up-regulated in the wild type plants as compared to *eds1-2*, also were detectable in two repetitions of the LC-MS/MS analysis. These were receptor serine/threonine kinase-like protein (*At*AED12 – *At4G18250*) and NIT1, indole-3-acetonitrile hydratase/nitrilase (*At*AED18 – *At3G44310*) (Table 22).

Summing up, by using three different proteomic approaches we identified 21 proteins, which accumulate in the apoplast of AvrRpm1-expressing wild type *Arabidopsis* plants in an *EDS1*-dependent manner. Among these 21 proteins are several proteins with a high

potential to be SAR-involved. In this respect, it is of interest that *At*AED12 and *At*AED18 were found by two independent methods. Moreover, *At*AED14, a PNP (plant natriuretic peptide A), is described in literature as a part of a class of systemically mobile signals (Ruzvidzo, Donaldson et al. 2011) (Wang, Gehring et al. 2011). This thesis focused on the three legume lectin-like proteins (*At*AED9-1, *At*AED9-2 and *At*AED9-3) due to their identification in two proteomic approaches and their literature-based characteristics described below.

4.2 The AED proteins – roles in plants

Among the AED proteins detected via the 2D-gel approach (Vlot *et al*., conducted at the Max Planck Institute for Plant Breeding Research, Cologne, Germany), three aspartyl protease family proteins were detectable (*At*AED1-3). They are hypothesized to play a role in proteolysis and expected to be located mostly in the apoplast as well as in the plant cell wall. *AtAED1* (*At5G10760*) encodes a predicted apoplastic aspartic protease that is unrelated to CDR1. Expression of *AtAED1* is induced by pathogen infection both in the infected and in the systemic leaves (Vlot, Parker, unpublished). Over expression of *AtAED1* in *Arabidopsis* represses SAR, indicating that *At*AED1 may function as part of a negative feedback loop controlling SAR (Wenig, Knappe, Vlot, unpublished). The second identified aspartic protease, *At*AED2, is preferentially expressed in *Arabidopsis* guard cells (Leonhardt, Kwak et al. 2004) and confers drought avoidance in *Arabidopsis* (Yao, Xiong et al. 2012). In the same publication it was named as ASPG1, which stands for Aspartic Protease in Guard Cell 1. Relatively little is known about *At*AED3, but it was characterized as a membrane-associated protein in *Arabidopsis* (de Jong, van Breukelen et al. 2006).

*At*AED4 and *At*AED5 are both predicted lipases, which are located mainly in the apoplast and are expected to play a role in the lipid metabolic process. Both corresponding genes belong to the GDSL lipase gene family containing more than 100 members. The gene structures among these members display a huge diversity (Ling 2008). Proteomic analysis of the culture fluid of *Arabidopsis* suspension cells treated with SA resulted in the identification of a secreted lipase with a GDSL motif, GDSL LIPASE 1 (GLIP1). Characterization of a *glip1* knock out (KO) mutant showed that GLIP1 likely is involved in the induction of systemic resistance signaling in *Arabidopsis* plants after challenge with the necrotrophic pathogen *Alternaria brassicicola* (Oh, Park et al. 2005). Previous work by others identified *At*AED4 as a lipid-binding protein in a proteomic analysis of *Arabidopsis* phloem exudates (Guelette, Benning et al. 2012). Consequently, the protein is hypothesized to be involved in phloem-mediated long-distance lipid signaling in plants (Benning, Tamot et al. 2012). The exact role of *At*AED5 is not characterized to date, but

due to the similarity in various aspects with *At*AED4, a comparable role can be hypothesized. This thesis showed that *At*AED4 and *At*AED5 together may be mobile in tobacco leaf tissue (Figure 37). Taken together, the data support a hypothesis where *At*AED4 and *At*AED5 may interact or cooperate to support long distance movement of an unknown lipid substrate triggering SAR.

Two actors in the carbohydrate metabolic process are *At*AED6 and *At*AED7, α-galactosidase and β-xylosidase. Interestingly, *At*AED6 was found in a proteomic approach to apoplastic proteins involved in cell wall regeneration. This study was carried out in *Arabidopsis* suspension cells (Kwon, Yokoyama et al. 2005). *At*AED7 was identified and characterized as an enzyme exhibiting β-D-Xylosidase activities in stem tissues of *Arabidopsis* (Minic, Rihouey et al. 2004).

Three additional AED proteins were detected via LC-MS/MS. Of these, the most promising one for my study, Legume Lectin Family Protein (*At*AED9-1), will be separately discussed below. In addition, *At*AED8 is located in the cell wall and mainly involved in the carbohydrate metabolic process. Its estimated location and function are closely related to those of *At*AED7. Glutamate synthase 1 (*At*AED10) encodes a gene whose sequence is similar to ferredoxin dependent glutamate synthase (Fd-GOGAT) (Ishizaki, Ohsumi et al. 2009) (Kissen, Winge et al. 2010). Expression in leaves is induced by light and sucrose. Moreover, *At*AED10 is proposed to be involved in photorespiration and nitrogen assimilation (Jamai, Salome et al. 2009). Its subcellular location is most likely chloroplastic, indicating that *At*AED10 may be a cytosolic contaminant of the apoplast extraction procedure.

The remaining 11 proteins were detected via quantitative ICPL analysis. *At*AED11 and *At*AED13 are both well known pathogenesis-related proteins, PR2 and PR5, which are involved in responses to pathogens. *At*AED12 is a member of the Arabidopsis Osmotin/thaumatin like superfamily of proteins consisting of 31 members. *At*AED12 is hypothesized to contain a protein-kinase like domain, being one among three proteins in that superfamily containing this domain (Abdin, Kiran et al. 2011).
The hypothetical protein named *At*AED14 encodes a plant natriuretic peptide A (PNP-A). PNPs are a class of systemically mobile molecules distantly related to expansins; their biological role has remained elusive. PNP-A contains a signal peptide domain and is secreted into the extracellular space. Co-expression analyses using microarray data suggest that PNP-A may function as a component of plant defense responses and SAR in particular. Therefore, PNP-A was classified as a newly identified PR protein (Ruzvidzo, Donaldson et al. 2011) (Wang, Gehring et al. 2011). *At*AED15 represents a chitinase,

which is involved in carbohydrate metabolic processes and mainly located in the apoplast and the plant cell wall.

*At*AED16 is a pectin methylesterase, targeted by a cellulose binding protein (CBP) from the parasitic nematode *Heterodera schachtii* during parasitism (Hewezi, Howe et al. 2008). *At*GSTF2, named *At*AED17 in this study, is a glutathione transferase protein belonging to the phi class of GSTs with a high functional diversity. *At*AED19 is known as EP1 and is SA sensitive (Blanco, Garreton et al. 2005).

Finally, *At*AED3, *At*AED6, *At*AED7, *At*AED8, all *At*AED9 proteins, *At*AED11, *At*AED13, *At*AED14 and *At*AED16 were identified in a mass-spectrometry-based proteomic approach as weakly bound cell wall proteins in apoplastic fluids of *Arabidopsis* rosettes (Boudart, Jamet et al. 2005). This means that 11 out of 21 in our study identified proteins were also detectable in a study, which dealt with apoplastic fluids of *Arabidopsis*. Another study analyzed the secretome of *Arabidopis* cell cultures via iTRAQ, a quantitative proteomics approach. The cell cultures were infected with three different *Pst* strains, including *Pst AvrRpm1* (Kaffarnik, Jones et al. 2009). Since the secretome of cell cultures basically represents the apoplast, the list of *At*AEDs was cross-referenced with the data presented by Kaffarnik, Jones et al. 2009. As a result, only *At*AED15 was detected and classified as induced by MAMPs/PAMPs.

4.3 Plant lectins and the legume-lectin-like protein family

Plant lectins were originally defined as proteins, which possess a high affinity to bind to carbohydrates, e.g. glycans of glycoproteins, glycolipids or polysaccharides (Goldstein and Hayes 1978). A more recent paper defines lectins as proteins that possess at least one non-catalytic domain that binds reversibly to a specific mono- or oligosaccharide (Peumans and Van Damme 1995). Virtually all plant lectins are classified based on their CRD (carbohydrate recognition domain) into 12 families (Van Damme, Lannoo et al. 2008). Due to their high binding affinity, they are able to serve as recognition molecules within a cell, between cells or even between organisms. Many plant lectins have been characterized so far, describing most of them as secretory proteins. This means that they can enter the secretory system and subsequently accumulate either in the vacuoles of cells or in the cell wall and the intercellular space (Chrispeels and Raikhel 1991). It is suggested that plant lectins may facilitate the wound response (Gibson, Stack et al. 1982) (Hamblin and Kent 1973), may act synergistically with chitinases to inhibit fungal growth (Broekaert, J et al. 1989) (Lerner and Raikhel 1992), or may function as feeding deterrents against insects (Melander, Ahman et al. 2003) (Sadeghi, Smagghe et al. 2008). Furthermore, it is believed that the most likely function of lectins is within plant defense (Van Damme, Barre et al. 2004) (Chrispeels and Raikhel 1991).

In the present study a protein family was identified to potentially play a role in SAR-induction. The family is called legume-lectin-like protein family. This family represents a class of homologous carbohydrate binding proteins that are mainly found in the seeds of most legume plants. Their quaternary structures and carbohydrate specificities vary widely, in contrast to their similarity on the level of their amino acid sequences as well as their tertiary structures (Loris, Hamelryck et al. 1998). The legume-lectin-like family consists of 18 members[1]. Two thereof have been identified in this study using LC-MS/MS and ICPL. Another one (*At*AED9-2) is 89% similar to the legume lectin-like protein identified by LC-MS/MS (*At*AED9-1).

The three lectins studied here are named *At*AED9-1 (*Arabidopsis thaliana* Apoplastic, *EDS1*-dependent protein 9-1; Legume lectin family protein; *At*3G15356), *At*AED9-2 (Lectin-like protein; *At*3G16530) and *At*AED9-3 (Legume lectin family protein, *At*5G03350).

It is reported that *AtAED9-1* (also named *AtLEC*) is up-regulated within hours by multiple stimuli including methyl jasmonate, ET, and the fungal elicitor chitin (Lyou, Park et al. 2009) (Jung, Lyou et al. 2007) (Jung, Yeu et al. 2007). Moreover, *At*AED9-1 accumulates after treatment of plants with oligogalacturonides elicitors released from the homogalacturonan of the plant cell wall during an attack by pathogenic micro-organisms (Casasoli, Spadoni et al. 2008). The oligogalacturonides elicitors represent a class of so called damage-associated-molecular-patterns (DAMPs) (Boller and Felix 2009). Because chitin induced *AtLEC* transcript accumulation also in JA and ET insensitive mutants, it appears that chitin promotes *AtLEC*/*AtAED9-1* expression via a JA/ET-independent pathway (Lyou, Park et al. 2009). Thus, *AtLEC*/*AtAED9-1* expression may be triggered by multiple different (defense) signaling pathways.

AtAED9-2 is rapidly and strongly induced by chitin (Zhang, Ramonell et al. 2002). Moreover, it is among 19 genes found in microarray experiments to be differentially expressed in SA-treated non-transgenic versus *2b*-transgenic Arabidopsis plants. The *Cucumber mosaic virus* (CMV) 2b counter-defense protein disrupts plant antiviral mechanisms mediated by RNA silencing and SA. *AtAED9-2* accumulated in *2b*-transgenic plants after SA treatment (Lewsey, Murphy et al. 2010), which leads to the assumption that *AtAED9-2* is not SA-inducible. In addition, *AtAED9-2* shows an an altered expression

[1] Poster: ARMIJO ET AL., FUNCTIONAL ANALYSIS OF LLP, A LECTIN LIKE PROTEIN INDUCED BY SALICYLIC ACID IN ARABIDOPSIS THALIANA AND INVOLVED IN THE DEFENSE RESPONSE AGAINST PSEUDOMONAS SYRINGAE; PRESENTED ON THE ICAR 2011 (INTERNATIONAL CONFERENCE ON ARABIDOPSIS RESEARCH) IN MADISON, WISCONSIN, USA

in a microarray experiment subsequent to the treatment of *Arabidopsis* with the aphid *Myzus persicae* (Couldridge, Newbury et al. 2007).

AtAED9-3 encodes another lectin that is located in the apoplast, the cell wall and plasma membrane (Armijo, García et al. 2011), or the chloroplast (Boudart, Jamet et al. 2005). Moreover, *AtAED9-3* was identified as being the gene with the highest level of activation after SA-treament of *Arabidopsis* plants in a micro-array experiment (Armijo, García et al. 2011). The biological role of *At*AED9-3 is not clear to date, but at least two earlier reports also found the corresponding gene to be the most induced gene after SA treatment of *Arabidopsis* suspension cells (Krinke, Ruelland et al. 2007) and whole plants (Blanco, Salinas et al. 2009). Moreover, it is up-regulated in six different *Arabidopsis* ecotypes after infections with Tobacco etch potyvirus (TEV) (Hillung, Cuevas et al. 2012).

All three candidate SAR signaling genes are up-regulated by *Brevicoryne brassicae* (cabbage aphid) during the entire infestation period on *Arabidopsis*. Timing and dynamics of early *Arabidopsis* responses to *B. brassicae* were measured (Kusnierczyk, Winge et al. 2008), which indicated a function of the *AtAED9* genes after herbivorous attack. In order to evaluate, if our *At*AED9 proteins are involved in plant-insect-interaction, experiments were carried out as part of this thesis work at the Boyce Thompson Institute, Cornell University, Ithaca, NY, USA in the lab of Prof. Georg Jander. In the first experiment *aed9-1* and *aed9-3* in comparison to *Arabidopsis* wild type plants were used for caterpillar (*Spodoptera exigua*) feeding experiments. Two larvae of *S. exigua* were put on each plant (no-choice-experiment) and caged. Their dry weight was measured 8 days after feeding. No significant difference was detectable between wild type and both KO lines. Another experiment was performed using the phloem-sucking aphid *Myzus persicae* (green peach aphid). One aphid per plant was used, caged and one day after feeding all progeny except one aphid was removed. Hence, a similar age of the aphids on each plant was secured. 8 days later the number of progeny was counted. Since again no differences were detected, *At*AED9-1 and *At*AED9-3 do not appear to affect the response of *Arabidopsis* to insects.

Due to their similarity to legume lectins, the lectin receptor kinases (lecRK) are mentioned very briefly. Both structural alignments and molecular modeling revealed striking similarities between the lectin-like domain of lecRK and related *A. thaliana* soluble lectins and legume lectins (Barre, Herve et al. 2002). LecRK should be unable to bind the simple sugars usually recognized by genuine legume lectins. Molecular modeling of the kinase domain suggests that, except for two apparently inactive receptors, all

other lecRK contain a putative functional Ser/Thr kinase catalytic domain (Barre, Herve et al. 2002).
Multiple *LecRKs* appear to be induced upon treatment of *Arabidopsis* with elicitors and by pathogen infection. The lectin receptor kinase LecRK-I.9 is a putative mediator of cell wall and plasma membrane adhesions in Arabidopsis. It is known to bind *in vitro* to the *Phytophtora infestans* effector IPI-O (Bouwmeester, de Sain et al. 2011). Another lectin-receptor-kinase, lectin-receptor-kinase 1, was reported to function during *Manduca sexta* herbivory to suppress the insect-mediated inhibition of JA-induced defense responses in *Nicotiana attenuata* (Gilardoni, Hettenhausen et al. 2011).

4.4 *AED* gene expression upon infection

The *AED* gene expression assay was performed to evaluate the behavior of the *AtAED* genes after pathogen attack. This assay resulted in (1) an overview of the regulation of the genes in response to infection of plants by virulent or avirulent pathogens and (2) insight into their dependency on *EDS1*.

The influence on *AED* gene expression was evaluated of four different treatments (MOCK (10mM $MgCl_2$), *Pst AvrRpm1*, *Pst AvrRps4* and *Pst*) in two plant lines (wild type and *eds1-2*) at two time points (two and three days after infection). As a control and reference the gene expression after MOCK treatment was evaluated (Figure 18). Six (*AtAED1*, *AtAED6*, *AtAED7*, *AtAED11*, *AtAED13* and *AtAED15*) out of 19 genes tested showed an induction already after infiltration of wild type *Arabidopsis* plants with 10mM $MgCl_2$. This can have different reasons. First, the genes are potentially induced due to certain environmental conditions (e.g. high light (Rossel, Wilson et al. 2002) or dehydration (Reymond, Weber et al. 2000)) or due to wounding while infiltrating the MOCK solution via a needless syringae into the leaf tissue. Upon wounding many genes are induced in *Arabidopsis*, most of them in a JA-dependent manner (Reymond, Weber et al. 2000). In the following, genes that show an up-regulation on top of the MOCK-induced induction are considered as up-regulated by the different bacterial strains tested.

Before the results of the gene expression experiments after bacterial infection are discussed, the relation between RPM1, RPS4 and EDS1 is clarified. Published data by Aarts, Metz et al. (1998) examined the relative requirements for *EDS1* and *NDR1* by a broad spectrum of *R* genes in three *Arabidopsis* accessions. They showed amongst others that *RPM1* is dependent on *NDR1*, whereas *RPS4* is dependent on *EDS1*. In contrast, they were not able to detect a dependency of *RPM1* on *EDS1*. Figure 43 illustrates the basic conclusions from the above mentioned paper, so that it can be transferred to our results.

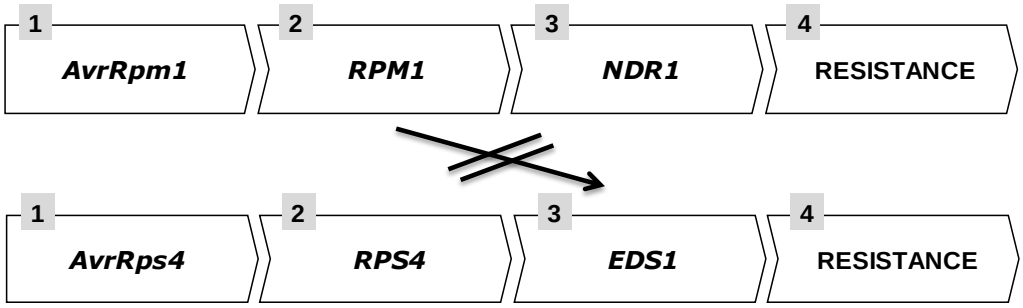


Figure 43: Simplified model to illustrate the relationship between *RPM1*, *RPS4* and *EDS1* and their connection leading to disease resistance (taken from (Aarts, Metz et al. 1998)

The scratched arrow indicates that there is no connection between *RPM1* and *EDS1*

Amongst the up-regulated genes upon infection of *Arabidopsis* with *Pst AvrRpm1* (Figure 19), the cluster of genes *AtAED11* (*PR2*) through *AtAED15*, is most striking. These are highly induced by *Pst AvrRpm1* mostly in an *EDS1*-independent manner. Four of these genes, *AtAED11* through *AtAED14*, are similarly induced by infection of *Arabidopsis* wild type plants with *Pst AvrRps4*. The latter induction is dependent on *EDS1*. Therefore, induction of *AtAED11* through *AtAED14* depends on *EDS1* if local resistance is dependent on *EDS1*, whereas expression of these genes also is triggered by the *NDR1*-supported defense pathway (Figure 43). Therefore, this set of genes may be generally involved in resistance processes and not necessarily specifically involved in SAR. This hypothesis is further supported by the mostly *EDS1*-dependent induction of *AtAED11* through *AtAED14* downstream from *Pst*, which triggers basal resistance in an *EDS1*-dependent manner. The same reasoning can be applied to *AtAED17*, induction of which is less pronounced upon infection but follows the same principle.

Two genes, *AtAED7* and *AtAED15*, are induced upon infection of *Arabidopsis* with any of the *Pst* strains tested. Their induction is independent of *EDS1* in all cases. This rationale also can be applied to *AtAED4*, albeit this gene is repressed by infection.

More interesting, however, are *AtAED1*, *AtAED6* and *AtAED9-3*, which are induced upon infection of *Arabidopsis* with any of the *Pst* strains tested at at least one time point after infection. Induction of this group of genes as well as repression of *AtAED5* is dependent on *EDS1* in all cases, meaning also upon infection of plants with *Pst AvrRpm1*. In addition, *AtAED9-1* and *AtAED9-2* are induced by *Pst AvrRpm1*, which at least in the case of *AtAED9-1* appears to be dependent on *EDS1*. Although local resistance (ETI) triggered by *Pst AvrRpm1* is independent of *EDS1* (Figure 43), *EDS1* is essential for SAR downstream from *Pst AvrRpm1* (Truman, Bennett et al. 2007). Thus, the regulation of

AtAED1, *AtAED5*, *AtAED6*, *AtAED9-1* and *AtAED9-3* appears to be highly correlated with SAR.

4.5 *AtAED9-1* and *AtAED9-2*: role in SAR

All experiments discussed in this section were performed using *Arabidopsis* wild type as well as *eds1-2* and *aed9-1* mutant plants. An *aed9-2* KO was not available, but *AtAED9-2* gene expression was included in the analysis. Generally, SAR experiments were conducted using either avirulent *Pst AvrRpm1* (Figure 23) or *Pst AvrRps4* (Figure 24) for the primary (1°) infection. The systemic tissue of primary infected plants was subsequently challenged with virulent *Pst*. Similar SAR experimental set-ups are routinely used in SAR research world wide (Attaran, Zeier et al. 2009) (Liu, Yang et al. 2010) (Liu, von Dahl et al. 2011).

Neither 1° inoculum, *Pst AvrRpm1* or *Pst AvrRps4*, triggered a reduction of growth of the challenge (2°) *Pst* inoculum in the systemic tissue of the *aed9-1* mutant (Figure 23; Figure 24). Thus, this mutant is SAR-deficient and we conclude that *AtAED9-1* may play an important role in proper SAR development. This finding must be confirmed by using an RNAi or overexpression line of the respective gene, because a second independent knock out line for *AtAED9-1* is not available. The detailed approach is described in "4.7 Over-expression and silencing of *AtAED9* genes in *Arabidopsis*: outcome" of this thesis.

Expression of the *AtAED9-1* and *AtAED9-2* genes was measured during a SAR experiment (see above) in *Arabidopsis* wild type plants in order to clarify a potential role in SAR induction and/or perception. After using *Pst AvrRpm1* as SAR-inducing bacteria, *AtAED9-1* and *AtAED9-2* were induced one day after pathogen infection in the infected tissue. This induction rapidly went down at three days after infection (Figure 30). In the gene expression analysis shown in Figure 19, expression of both *AtAED9-1* and *AtAED9-2* was at a lower level already at two days after infection as compared to one day after infection in the experiment shown in Figure 30. Therefore, both genes are induced relatively early at one day after *Pst AvrRpm1* infection with their expression returning to basal levels within two to three days after infection. By contrast, *AtAED9-1* and *AtAED9-2* are not induced systemically (Figure 31). Together, the data point towards a potential involvement of *AtAED9-1* and *AtAED9-2* in the SAR signal generating tissue (1°) rather than in SAR signal perception systemically (2°).

AtAED9-2 is induced by *Pst AvrRpm1* in the infected tissue one day after infection of the *aed9-1* KO mutant (Figure 32). This induction is not stronger than in wild type plants and

the endogenous *AtAED9-2* gene is not sufficient to support SAR in the *aed9-1* mutant. Therefore, in spite of the high similarity between *AtAED9-1* and *AtAED9-2* (89%) and in spite of their coregulation upon infection of wild type plants, both genes may perform separate functions during SAR. To decipher the role in SAR of *AtAED9-2* an overexpression line is needed and the performance of this line should be tested in a SAR experiment. Furthermore, an RNAi line targeting *AtAED9-2* should be generated. As this work focused on the interplay between the *AtAED9* genes, RNAi lines were prepared targeting either both *AtAED9-1* and *AtAED9-2* or all three *AtAED9* genes for silencing. The detailed approach is described in "4.7 Over-expression and silencing of *AtAED9* genes in *Arabidopsis*: outcome".

Although SAR triggered by infection of plants with *Pst AvrRps4* is abolished in the *aed9-1* mutant, expression of *AtAED9-1* and *AtAED9-2* was not induced by this pathogen in the primary infected or systemic tissues at any of the time points tested. This could indicate that (1) *AtAED9-1* and/or *AtAED9-2* expression is induced at earlier or later time points, which were not measured in this thesis or (2) that a heightened expression of *AtAED9-1* and/or *AtAED9-2* is not needed to establish SAR after *Pst AvrRps4* infection.

As the SAR defect of the *aed9-1* mutant could be caused by an aberrant immune response to avirulent *Pst* in the primary infected tissue, growth was analyzed of the avirulent *Pst* strains mentioned above as well as of virulent *Pst* (Figure 25 - Figure 29). Growth of all strains was similar in *aed9-1* as compared to wild type plants. The *eds1* mutant displays a compromised defense response against *Pst AvrRps4* and *Pst*, resulting in enhanced growth of these bacteria in *eds1* mutant as compared to wild type plants. This was not detected in *aed9-1*. Therefore, it is hypothesized that *AtAED9-1* is not needed in the primary infected tissue to initiate local defense responses to avirulent or virulent bacteria. Taken together, the data indicate that *AtAED9-1* may be specifically involved in SAR, since SAR, but not PTI and ETI, is abolished in the *aed9-1* mutant.

4.6 *AtAED9-3*: role in SAR

All the experiments discussed in this section were performed using *Arabidopsis* wild type plants and the *eds1-2* and *aed9-3* mutants. SAR experiments discussed here were conducted as in the previous section.

Neither 1° inoculum, *Pst AvrRpm1* or *Pst AvrRps4*, triggered a significant reduction of growth of the challenge (2°) *Pst* inoculum in the systemic tissue of the *aed9-3* mutant (Figure 23; Figure 24). Thus, this mutant is SAR-deficient and we conclude that

AtAED9-3, similar to *AtAED9-1*, may play an important role in proper SAR development. This finding can and must be confirmed by using a second independent knock out line for *AtAED9-3.*

Similar to *AtAED9-1*, *AtAED9-3* appears to be exclusively involved in SAR because local immune responses to *Pst AvrRpm1, Pst AvrRps4* and *Pst* were normal as detected by growth analysis of the bacteria in the *aed9-3* mutant as compared to wild type plants (Figure 27,Figure 28, and Figure 29).

In terms of gene expression during SAR, *AtAED9-3* is not induced locally upon *Pst AvrRpm1* primary infection (1°) as compared to MOCK-treated wild type *Arabidopsis* plants. By contrast, the expression of *AtAED9-3* is induced in the systemic, SAR-induced tissue (2°) at both time points analyzed (1dai and 3dai). This indicates a potential involvement of *AtAED9-3* in SAR in the systemic, SAR signal perceiving tissue (2°). Subsequent to the usage of *Pst AvrRps4* as SAR-inducing bacteria, expression of *AtAED9-3* was induced also in the primary infected tissue (1°) one day after infection as compared to MOCK-treated plants (Figure 33). This induction went down at three days after infection (Figure 20 and Figure 33), indicating that *AtAED9-3*, similar to *AtAED9-1* and *AtAED9-2*, is a fast-induced gene. Systemically (2°), the expression of *AtAED9-3* was induced at both time points analyzed (1dai and 3 dai), if the primary tissue (1°) had been treated with *Pst AvrRps4* (Figure 34). The up-regulation in the systemic tissue (2°) was even enhanced three days after infection of the primary infected tissue (1°) as compared to one day after infection. Thus, in contrast to *AtAED9-1* and *AtAED9-2*, *AtAED9-3* represents a gene that is induced after *Pst AvrRps4* treatment.

Taken the last two passages together, *AtAED9-1* and *AtAED9-2* showed similar expression patterns, whereas *AtAED9-3* expression was different. This fits to their similarity based on their coding (Figure 22) and protein sequences (Figure 45), where *AtAED9-1* and *AtAED9-2* displayed a closer homology to each other than to *AtAED9-3*. Based on its local and systemic induction *AtAED9-3* may play a role in SAR induced with *Pst AvrRps4*, even if the *aed9-3* KO mutant exhibits a statistically insignificant tendency towards SAR. SAR in response to *Pst AvrRpm1* was fully abolished in the *aed9-3* mutant. By contrast, induction of *AtAED9-1* and *AtAED9-2* seems to be restricted to tissues infected with *Pst AvrRpm1.* A reaction to *Pst AvrRps4* was not detected in the infected or systemic tissue. Nevertheless, SAR triggered by either *Pst AvrRpm1* or *Pst AvrRps4* was abolished in the *aed9-1* mutant. Together, the data show that both *AtAED9-1* and *AtAED9-3* are involved in SAR, but not local resistance against virulent or avirulent *Pst*. Strikingly, expression of *AtAED9-1* and *AtAED9-2* is restricted to the local infected tissue

in response to *Pst AvrRpm1*, whereas expression of *AtAED9-3* appears to be mainly systemic in response to both primary inoculi applied in this study.

4.7 Over-expression and silencing of *AtAED9* genes in *Arabidopsis*: outcome

Over-expression and silencing lines of the *AtAED9* genes were generated to allow a more thorough characterization of the role of the *AtAED9* family in SAR. This was necessary, because (1) no KO mutant is available for *AtAED9-2*, (2) only one KO mutant is available for *AtAED9-1*, and (3) we analyzed only a single KO mutant for *AtAED9-3*. Potential roles in SAR of *AtAED9* family members as shown in this thesis must be confirmed by at least one additional independent approach. The integrated approach suggested here includes over expression, RNAi, and KO lines and is widely applied in plant research, e.g. to understand the role of the senescence-related *WRKY53* transcription factor (Miao, Laun et al. 2004). At this point more examples of this widely used approach are not discussed.

The *AtAED9* silencing strategy was based on the incorporation of RNAi constructs in wild type and *eds1-2* mutant *Arabidopsis* plants. RNAi is also known as post-transcriptional gene silencing (Hannon 2002) and is widely applied to silence the expression of a specific gene if the KO is lethal or if no KO is available. Moreover, RNAi has the advantage that multiple genes can be silenced at the same time. *AtAED9-1* shares a high similarity with *AtAED9-2*. Thus, one RNAi construct was designed to target both *AtAED9-1* and *AtAED9-2* (double silencing construct). The strategy to silence *AtAED9-2* was not followed up further in this work, but will be essential to decipher a role in immunity and/or SAR of this gene. A second RNAi construct aimed to knock down all three *AtAED9* genes (triple silencing construct). qRT-PCR analysis of transformed and selected F1 plants has shown that the *AtAED9* genes can be silenced in both wild type and *eds1-2* genetic backgrounds (Pabst and Vlot, follow-up work to this thesis). Strikingly, silencing of all three *AtAED9* genes by the triple silencing construct is more efficient than silencing of *AtAED9-1* and *AtAED9-2* alone, although the sequences targeting *AtAED9-1* and *AtAED9-2* are the same in the double and triple silencing constructs. Also, the double and triple silencing constructs are more effective in wild type plants than in the *eds1-2* mutant background. These observations lead to the hypothesis that the *At*AED9 proteins may functionally interact with each other and with EDS1. Alternatively, *At*AED9-3 may obstruct efficient silencing of *AtAED9-1*/*9-2* if the presence of *At*AED9-3 alone is harmful to the plant. Further selection steps need to be applied to obtain homozygous transgenic plants, the performance of which can be evaluated in SAR experiments and other analyses. These should shed more light on possible functions of the *At*AED9 family in immunity and SAR as well as on functional interactions within the *At*AED9 family and with EDS1.

With the purpose to overexpress the three *AtAED9* genes in *Arabidopsis* wild type and *eds1-2* plants, overexpression constructs were cloned using the expression vector pER8-GW-Cterm-3XHA Strep (Table 4) with an estradiol-inducible promoter. After transformation of the constructs into *A. tumefaciens* strain GV3101, plants were transformed using the floral dipping method (Clough and Bent 1998). After the selection of F1 seeds on antibiotic-containing media, all emerging plants died. This can be caused by a potential 'leakage' of the estradiol-inducible promoter leading to elevated accumulation of *At*AED9 proteins, which might be lethal to the plant. In support of a possible toxic effect of the *At*AED9 proteins it also was observed that *A. tumefaciens* carrying the expression vector harboring any of the *AtAED9* coding sequences needed a prolonged incubation time for proper growth as compared to other *A. tumefaciens* clones. *A. tumefaciens* carrying an *AtAED9* construct required 3X O/N, whereas Agrobacteria normally just need 2X O/N for proper multiplication. This would imply that *AtAED9* was expressed from a 'leaky' estradiol-inducible promoter in both *Arabidopsis* and *A. tumefaciens.*

4.8 Lectins in stress response/tolerance

Potential biological roles of lectins, including of the *At*AED9 proteins, are reported in insect resistance and/or sugar signaling. The potential functions of lectins in these processes are discussed in this section and could (1) explain the hypothesized toxicity of the *At*AED9 proteins in the overexpression lines and (2) provide ideas on the potential function of the *At*AED9 proteins during defense against pathogens, including SAR.

There is still a controverse discussion about the biological role of lectins in their parent organisms. Lectins are for instance able to bind to small plant growth regulators such as adenine (Roberts and Goldstein 1983), they play a role in plant defense mechanisms (Chrispeels and Raikhel 1991) (Peumans and Van Damme 1995) and they are highly toxic when ingested by mammals as they survive gastrointestinal passage (Vasconcelos and Oliveira 2004) (Pusztai, Ewen et al. 1993). The toxicity of lectins is well described in plant-insect-interactions, where various lectins have shown entomotoxic effects when fed to insects (Vandenborre, Smagghe et al. 2011) (Powell 2001) (Machuka, Van Damme et al. 1999) (Zhu-Salzman, Shade et al. 1998). Lectins were shown to have an effect on insects via binding to the brush-border membrane of the intestinal epithelial cells of the insect or, in the case of chitin-binding lectins, to the peritrophic membrane (Peumans and Van Damme 1995) (Chrispeels and Raikhel 1991). In plants, toxic effects of lectins have not been described to date, but can be expected e.g. due to their defense-inducing capacity in response to PAMPs such as chitin (Lyou, Park et al. 2009) (Zhang, Ramonell

et al. 2002). If elevated lectin levels were to trigger constitutive SA-dependent defenses, this would likely result in severely dwarfed plants or, in extreme cases, in death. Hence, it can only be speculated about the role of the *At*AED9 proteins after overexpression in *Arabidopsis* plants. It could be that the total amount of lectins was too high resulting in constitutive SA-dependent defenses or in lethality due to interactions of lectins with endogenous plant growth regulators. In order to overcome these potential effects, overexpression of the *AtAED9* genes can possibly be achieved in transgenic plants carrying additional copies of the respective *AtAED9* coding sequences downstream from their own native promoters. In the case of *AtAED9-1* and *AtAED9-3*, the same constructs also can be used to complement the respective knock-out lines *aed9-1* and *aed9-3* (Table 1). Using the native promoters to drive *AtAED9* gene expression would ensure that the accumulation of the gene products follows the natural spatio-temporal distribution of the endogenous *AtAED9* gene products.

All three *At*AED9 proteins belong to the legume lectin-like protein family, indicating a similar physiological behavior. It is known that lectins are involved in sugar binding processes. Sugars are recognized as important regulatory molecules with signal functions in plants and other organisms (Rolland, Baena-Gonzalez et al. 2006). By binding specific sugars, e.g. glucoses, the overall sugar composition in the phloem could be affected/changed and hence e.g. lead to PR protein accumulation (Xiao, Sheen et al. 2000), which is closely related to SAR. It has been shown that sucrose is involved in *PR2* gene expression (Thibaud, Gineste et al. 2004). Furthermore, a study conducted in the mid-1990´s found that hexose sensing for defense gene activation may occur at secretory membranes (Herbers, Meuwly et al. 1996). The same authors also found that a certain threshold level of hexoses is required for defense gene activation, but also that the leaf developmental stage is important (Herbers, Meuwly et al. 1996). In addition, it was shown in rice that two different hexokinases *Os*HXK5 and *Os*HXK6 are important sugar sensors (Cho, Ryoo et al. 2009), but also other hexokinases play a role in sugar signaling/sensoring (Rolland, Baena-Gonzalez et al. 2006) (Jang, Leon et al. 1997). Another *Arabidopsis* glucose sensor HXK1 is involved in light, nutrient and hormonal signaling, including plant growth hormones such as auxin, gibberellins, and brassinosteroids (Moore, Zhou et al. 2003). Taken together, hexose sensing e.g. by hexokinases may be an important regulator of immunity in plants. Here, we hypothesize that the functions in SAR of the potentially sugar-binding *At*AED9 proteins may be related to sugar sensing in general. This hypothesis is supported by the predicted localization of the *At*AED9 proteins, which is mainly in the apoplast and the cell wall. Interestingly, three other AED proteins, *At*AED6, *At*AED7 and *At*AED8, are predicted to be involved in carbohydrate metabolism and could therefore be functionally linked to the *At*AED9 proteins during SAR.

A third and last potential function of the *At*AED9 proteins could be in the generation of so-called endogenous elicitors. In addition to elicitor molecules derived from attacking organisms, endogenous elicitors are derived from the plant itself (Yamaguchi and Huffaker 2011). The lectins could potentially bind to such endogenous elicitors and after pathogen attack, release these elicitors to amplify the plants defense response.

4.9 Systemic TMV assay

The systemic tobacco TMV assay was developed combining two methods: transient over-expression of a gene-of-interest in the local tissue (1°) followed by an infection of the systemic tissue (2°) using TMV.

Transient expression of diverse proteins is widely used in plant molecular biology, e.g. for HR induction by proteins upon their transient expression by agroinfiltration (Bendahmane, Querci et al. 2000) or for fluorescent fusion protein expression in suspension cells (Miao and Jiang 2007). The use of the tobacco-TMV-system to elucidate the mechanisms underlying SAR goes back until 1961 (Ross 1961), but is still widely applied (Pallas, Paiva et al. 1996) (Liu, von Dahl et al. 2011).
To successfully combine both methods, various pre-experiments were conducted in order to determine the optimal time points for sampling and infection. AED proteins were expressed using *A. tumefaciens*-mediated transient expression (agroinfiltration) (Bendahmane, Querci et al. 2000). Western blot analysis of the agroinfiltrated tissue (1°) (Figure 37) revealed that all proteins were expressed 60 hours after infiltration. Protein expression was detectable in the infiltrated, but not in the uninfiltrated regions of the leaves. In one experiment AED protein accumulation could be detected also in the uninfiltrated region of the agroinfiltrated leaf upon combined expression of *At*AED4 and *At*AED5. Therefore, these proteins together may be mobile within the leaf as compared to the application of either *At*AED4 or *At*AED5 alone (Figure 37). This could be due to dimerization of both proteins. Alternatively, each protein alone might be mobile, but escaping detection in our analyses. In this respect, it should be noted that protein accumulation was extremely high in the infiltrated parts of agroinfiltrated leaves in the experiment, in which the mobility of proteins was detected. Although this observation was not reproduced, *At*AED4 also was hypothesized by others to be mobile, in that case in the phloem (Benning, Tamot et al. 2012). Because *At*AED4 is a lipid-binding protein (Guelette, Benning et al. 2012), *At*AED4 and/or *At*AED5 may be involved in phloem-mediated long distance lipid signaling (Benning, Tamot et al. 2012).
Via the systemic TMV assay the SAR-inducing potential of transiently expressed *At*AED1, *At*AED4, *At*AED5 and *At*AED4/5 (1:1) was evaluated in two different tobacco lines. We

could show that the 1:1 mixture of *At*AED4 and *At*AED5 induced SAR in the systemic tissue against TMV after transient over expression of the proteins in the local, agroinfiltrated tissue (1°) of *Nicotiana tabacum* (tobacco) Xanthi nc (Figure 40). In contrast, *At*AED1 appears to have an inhibitory effect on proper SAR development, which was shown by the inhibition of *PR1* expression in the bordering tissue (B) of agroinfiltrated zones. This observation supports preliminary data in transgenic *Arabidopsis* plants, in which conditional over expression of *At*AED1 inhibits SAR (Wenig, Knappe, and Vlot, unpublished).

*At*AED4 and *At*AED5 each individually triggered SAR in 50% of the plants tested. Since both proteins together always triggered SAR, it can be hypothesized that *At*AED4 and *At*AED5 interact either directly (heterodimer formation) or indirectly to amplify the SAR signaling process. It is possible that both proteins are active in parallel or in succession to trigger SAR. SAR triggered by *At*AED4 and/or *At*AED5 in the systemic TMV assay in tobacco appears to be true, SA-dependent SAR. Neither the individual proteins nor the combination of *At*AED4 and *At*AED5 triggered SAR in SA-degrading, *NahG* transgenic tobacco. Since *At*AED4 has been shown to bind lipids, which it may transport through the phloem, *At*AED4 and *At*AED5 might be involved in the transfer of a lipid long-distance signal triggering SA-dependent SAR. As we conclude about the function of *At*AED4 and *At*AED5 related to SAR in an artificial system, these results need to be confirmed in transgenic *Arabidopsis* plants over expressing *AtAED4* and/or *AtAED5*.

4.10 Conclusion and Outlook

In this thesis, 21 AED proteins were identified that are potentially related to SAR in *Arabidopsis* plants. To this end, an integrated proteomics approach was applied. Of the 21 AED proteins, the three members of the *AtAED9* gene family were characterized further in more detail. Using *aed9-1* and *aed9-3* KO mutants, it was shown that *AtAED9-1* and *AtAED9-3* are required for SAR, but not for local resistance during a primary infection. This was shown by bacterial growth curve experiments and SAR experiments, in which either *Pst AvrRpm1* or *Pst AvrRps4* was used as the primary, SAR-inducing inoculum. Furthermore, the results from the SAR experiments and the subsequent qRT-PCR analyses indicated that *AtAED9-1* and *AtAED9-2* might play a role in SAR in the primary infected tissue (1°). Although *AtAED9-3* may also be active in the primary infected (1°) tissue, it appears to be more prominently associated with SAR signaling in the systemic, SAR signal perceiving tissue (2°). Figure 44 represents a graphical summary of the hypotheses gained from the infection experiments (including SAR and

growth curve experiments) as well as from the parallel gene expression measurements performed in this thesis.

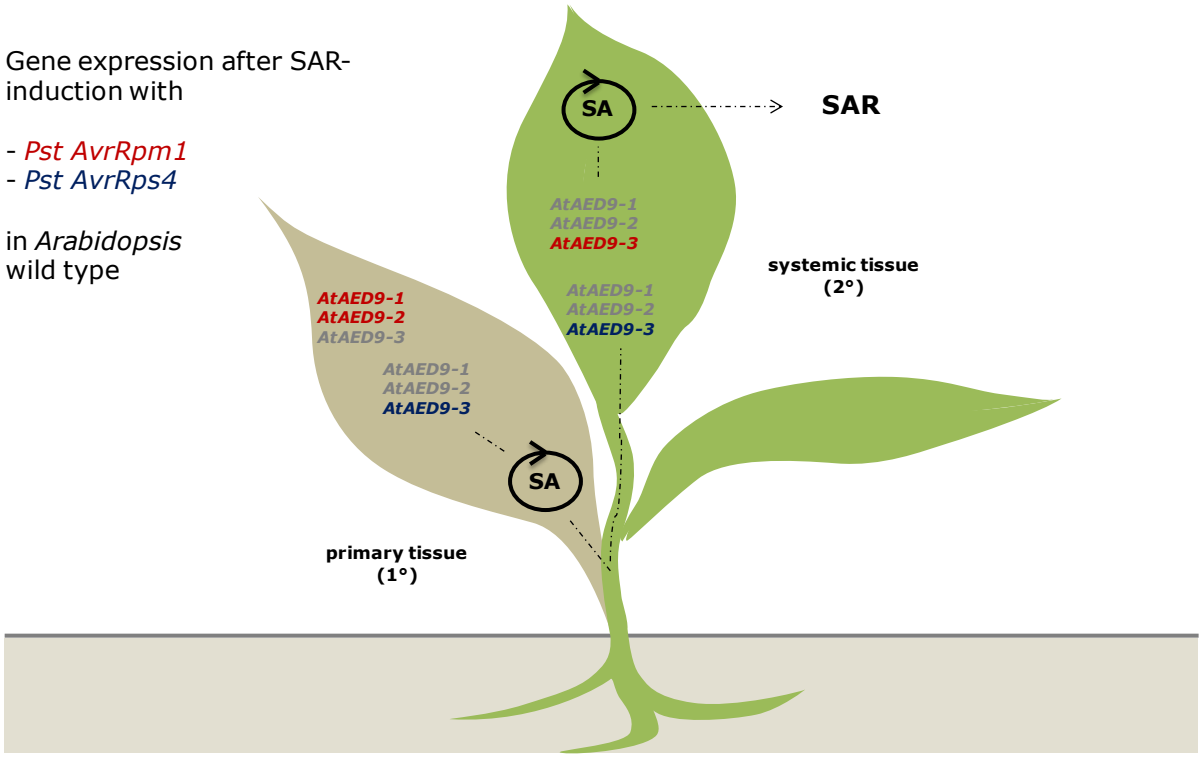


Figure 44: Working model.

This model illustrates the potential role of the AED9 proteins during SAR induced by two different bacterial strains. *AtAED9-1* and *AtAED9-2* are up-regulated in the primary tissue (1°) after infection with *Pst AvrRpm1*, whereas *AtAED9-3* is up-regulated after *Pst AvrRps4* treatment. This potentially leads to enhanced SA production, which in turn generates a mobile signal that is transported via the phloem to the systemic tissue (2°). The so generated SAR signal directly or indirectly leads to the up-regulation of *AtAED9-3* in the systemic tissue (2°). This would be followed by an enhanced SA production in the systemic tissue (2°), which subsequently leads to SAR.

Marked in red: SAR experiment (Figure 23), where SAR was induced with *Pst AvrRpm1* including the regulation of *AtAED9-1* through *AtAED9-3* in the local (1°) as well as systemic tissue (2°); genes that were up-regulated are highlighted in bold and red in the primary (1°) and systemic tissue (2°).

Marked in blue: SAR experiment (Figure 24), where SAR was induced with *Pst AvrRps4* including the regulation of *AtAED9-1* through *AtAED9-3* in the local (1°) as well as systemic tissue (2°); genes that were up-regulated are highlighted in bold and blue in the primary (1°) as well as in the systemic tissue (2°).

Marked in grey: Genes that are not regulated after treatment

Depending on the avirulent bacteria used in the primary tissue (1°) to initiate SAR, expression of either the combination of *AtAED9-1* and *AtAED9-2* (*Pst AvrRpm1*) or of *AtAED9-3* is induced (*Pst AvrRps4*) in the infected tissue. This induction may be part of a mechanism that leads to SAR signal generation. SAR signal(s) then move through the phloem to the systemic tissue (2°) and directly or indirectly enforce the expression of *AtAED9-3*, which may be involved in SAR signal perception and/or propagation. The induction of the *AtAED9* genes may be specifically related to SAR because basal resistance to the SAR-inducing avirulent bacteria or to the virulent challenge bacteria is not affected in the *aed9-1* and *aed9-3* KO mutants. As a future perspective, different

questions need to be answered in order to elucidate the detailed functions of the *At*AED9 proteins during SAR.

First, additional SAR experiments should be conducted using a second independent KO mutant line for *AtAED9-3* in order to confirm the SAR-deficient phenotype of the already tested KO line. Due to the fact that there is just one KO mutant line available for *AtAED9-1* and no mutant line for *AtAED9-2*, silencing lines need to be generated. As already shown in this thesis clones for such an approach were generated. A double silencing construct targeting *AtAED9-1* and *AtAED9-2* and a triple silencing construct targeting all three *AtAED9* genes was transformed into wild type and *eds1-2 Arabidopsis* plants. The selection of transgenic plants is ongoing. Using these plant lines can on the one hand provide information about the knock-down of *AtAED9-2* and on the other hand, and more importantly, about either the interplay between *AtAED9-1* and *AtAED9-2* (double silencing construct) or between all the *At*AED9 proteins (triple silencing construct). The questions that can be answered using this approach are: (1) Does silencing of multiple *AED9* genes, as opposed to single KO mutants, affect the plant's susceptibility to infection?, (2) Does silencing of multiple *AED9* genes further compromise SAR?, (3) Can *AtAED9-3* support SAR in the absence of both *AtAED9-1* and *AtAED9-2*?, and (4) Can further functional interactions between *AtAED9-1* and *AtAED9-2*, between all three *AtAED9* genes, and/or between *AtAED9-1/9-2* and *AtAED9-3* be detected? After the first selection steps of the transgenic plants it was shown that silencing of *AtAED9-1* and *AtAED9-2* was harder to achieve than silencing of all three *AtAED9* genes (Pabst and Vlot, follow-up work to this thesis). On top, silencing was easier in *Arabidopsis* wild type plants than in *eds1-2* mutants, indicating a potential connection between the *At*AED9 proteins and EDS1.

Another option to decipher a gene function is represented by over expression. As a result of the attempt to over express the *AtAED9* genes using the estradiol-inducible promoter in wild type and *eds1-2 Arabidopsis* plants, the transformed plants were not viable. A potential toxic effect of the legume lectin-like proteins was previously discussed in this work. In future, it should be tested if the *AtAED9* genes can be expressed from their own native promoters. If so, it would be of interest to test if such transgenic plants display enhanced resistance to pathogens. In addition, native promoter-driven constructs of *AtAED9-1* or *AtAED9-3* could be used for complementation studies of the corresponding KO mutants used in this thesis. Such complementation lines should theoretically show the *Arabidopsis* wild type phenotype, where SAR is functional. An interesting point to decipher would be if the whole protein is needed to establish a proper SAR development or only certain domains. A bioinformatics approach could establish conserved and/or potential functional domains in the members of the *At*AED9 family that could e.g. bind

carbohydrates or be responsible for a location in the cell wall. Subsequently, mutant versions of the genes, in which predicted functional domains are knocked out, can be expressed in the corresponding mutant backgrounds to test which domains are required for SAR. These studies would provide clues about the mechanism by which the *At*AED9 family affects SAR. Finally, GFP (*green fluorescent protein*) (Shimomura, Johnson et al. 1962), widely used as a marker protein, can be included in separate, native promoter-driven overexpression constructs. Subsequently, the subcellular localization of the *At*AED9 proteins can be elucidated. Using GFP-marked versions of AED9, questions could be addressed such as if the *At*AED9 proteins are released out of the cell after infection or if they are constitutively located in the cell wall.

As outlined above the *At*AED9 proteins are expected to have a sugar binding function. Interestingly, three other AEDs are predicted to be involved in carbohydrate metabolism in the cell wall (obtained from Arabidopsis.org). These are *At*AED6 (alpha-galactosidase), *At*AED7 (β-xylosidase) and *At*AED8 (glycosyl hydrolase family 3 protein). These proteins may affect the formation of glycan structures present in the cell wall or e.g. on proteins in the plasma membrane. Taken together, it can be hypothesized that *At*AED6, *At*AED7 and *At*AED8 cleave certain sugar domains, which are subsequently bound by the *At*AED9 proteins, which may in turn disrupt the sugar composition in apoplast, cell wall or phloem leading to SAR. In that light, it is of interest to note that an *aed7* KO mutant is SAR-deficient (Wenig, Baires, and Vlot, unpublished) and that preliminary data suggest that conditional over expression of *AtAED6* also compromises SAR (Wenig, Wittek, and Vlot, preliminary). In future, it would be of interest to study if, and if so how the members of the *At*AED9 family functionally interact with *At*AED6, *At*AED7 and/or *At*AED8. A first indication that the proteins may mechanistically cooperate during SAR could be gained from gene expression studies using e.g. *GFP* reporter gene constructs driven by the native promoters of the respective genes-of-interest. Spatio-temporal co-expression of the different genes, in particular during infection and SAR, would indicate a potential functional interaction. Further studies would then have to be conducted to establish more mechanistic details of this interaction/cooperation. For instance, yeast-two-hybrid analysis could provide clues about potential protein-protein interactions amongst the proteins-of-interest or with other proteins. In addition, recombinant proteins of the *At*AED9 family members could be isolated from *E. coli*. A binding assay with plant extracts could be used and subsequently be evaluated for metabolites and/or proteins. Using such an experimental set up, we might be able to clarify, which products are bound by the *At*AED9 proteins.

Together, the approaches outlined above would give a detailed insight into the functional properties of the *At*AED9 proteins and their relation to systemic acquired resistance. In this thesis it was shown that at least two *At*AED9 proteins are specifically involved in

SAR, but not in basal resistance to pathogens. Independent of their mode of action in the primary (1°) or systemic (2°) tissue, this is the first work that shows a connection between lectins and SAR in *Arabidopsis* plants.

Chapter 5: REFERENCES

Aarts, N., M. Metz, E. Holub, B. J. Staskawicz, M. J. Daniels and J. E. Parker (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**(17): 10306-10311.

Abdin, M. Z., U. Kiran and A. Alam (2011). "Analysis of osmotin, a PR protein as metabolic modulator in plants." Bioinformation **5**(8): 336-340.

Alfano, J. R. and A. Collmer (2004). "Type III secretion system effector proteins: double agents in bacterial disease and plant defense." Annu Rev Phytopathol **42**: 385-414.

Alonso, J. M., A. N. Stepanova, T. J. Leisse, C. J. Kim, H. Chen, P. Shinn, D. K. Stevenson, J. Zimmerman, P. Barajas, R. Cheuk, C. Gadrinab, C. Heller, A. Jeske, E. Koesema, C. C. Meyers, H. Parker, L. Prednis, Y. Ansari, N. Choy, H. Deen, M. Geralt, N. Hazari, E. Hom, M. Karnes, C. Mulholland, R. Ndubaku, I. Schmidt, P. Guzman, L. Aguilar-Henonin, M. Schmid, D. Weigel, D. E. Carter, T. Marchand, E. Risseeuw, D. Brogden, A. Zeko, W. L. Crosby, C. C. Berry and J. R. Ecker (2003). "Genome-wide insertional mutagenesis of Arabidopsis thaliana." Science **301**(5633): 653-657.

Anderson, J. P., E. Badruzsaufari, P. M. Schenk, J. M. Manners, O. J. Desmond, C. Ehlert, D. J. Maclean, P. R. Ebert and K. Kazan (2004). "Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis." Plant Cell **16**(12): 3460-3479.

Armijo, G., C. García, A. Seguel, L. Leon, P. Salinas, D. Leiva and L. Holuigue (2011). "Functional analysis of LLP, a lectin like protein induced by salicylic acid in Arabidopsis thaliana and involved in the defense response against Pseudomonas syringae." Conference Proceedings: 22ND INTERNATIONAL CONFERENCE ON ARABIDOPSIS RESEARCH **Publication:501746726**.

Asai, T., G. Tena, J. Plotnikova, M. R. Willmann, W. L. Chiu, L. Gomez-Gomez, T. Boller, F. M. Ausubel and J. Sheen (2002). "MAP kinase signalling cascade in Arabidopsis innate immunity." Nature **415**(6875): 977-983.

Attaran, E., T. E. Zeier, T. Griebel and J. Zeier (2009). "Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in Arabidopsis." Plant Cell **21**(3): 954-971.

Bari, R. and J. D. Jones (2009). "Role of plant hormones in plant defence responses." Plant Mol Biol **69**(4): 473-488.

Barre, A., C. Herve, B. Lescure and P. Rouge (2002). "Lectin receptor kinases in plants." Critical Reviews in Plant Sciences **21**(4): 379-399.

Bartsch, M., E. Gobbato, P. Bednarek, S. Debey, J. L. Schultze, J. Bautor and J. E. Parker (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**(4): 1038-1051.

Belkhadir, Y., R. Subramaniam and J. L. Dangl (2004). "Plant disease resistance protein signaling: NBS-LRR proteins and their partners." Curr Opin Plant Biol **7**(4): 391-399.

Bellafiore, S. and S. Briggs (2007). "Exploring plant-host compatibility effectors secreted by Meloidogyne incognita using a LC ESI MSMS proteomic approach." Phytopathology **97**(7): S9-S9.

Bendahmane, A., M. Querci, K. Kanyuka and D. C. Baulcombe (2000). "Agrobacterium transient expression system as a tool for the isolation of disease resistance genes: application to the Rx2 locus in potato." Plant Journal **21**(1): 73-81.

Bender, C. L., F. Alarcon-Chaidez and D. C. Gross (1999). "Pseudomonas syringae phytotoxins: Mode of action, regulation, and biosynthesis by peptide and polyketide synthetases." Microbiology and Molecular Biology Reviews **63**(2): 266-+.

Benning, U. F., B. Tamot, B. S. Guelette and S. Hoffmann-Benning (2012). "New aspects of Phloem-mediated long-distance lipid signaling in plants." Front Plant Sci **3**: 53.

Bent, A. F. (1996). "Plant Disease Resistance Genes: Function Meets Structure." Plant Cell **8**(10): 1757-1771.

Berrocal-Lobo, M. and A. Molina (2004). "Ethylene response factor 1 mediates Arabidopsis resistance to the soilborne fungus Fusarium oxysporum." Mol Plant Microbe Interact **17**(7): 763-770.

Bhattacharjee, S., M. K. Halane, S. H. Kim and W. Gassmann (2011). "Pathogen effectors target Arabidopsis EDS1 and alter its interactions with immune regulators." Science **334**(6061): 1405-1408.

Bindschedler, L. V., M. Palmblad and R. Cramer (2008). "Hydroponic isotope labelling of entire plants (HILEP) for quantitative plant proteomics; an oxidative stress case study." Phytochemistry **69**(10): 1962-1972.

Blanco, F., V. Garreton, N. Frey, C. Dominguez, T. Perez-Acle, D. Van der Straeten, X. Jordana and L. Holuigue (2005). "Identification of NPR1-dependent and independent genes early induced by salicylic acid treatment in Arabidopsis." Plant Mol Biol **59**(6): 927-944.

Blanco, F., P. Salinas, N. M. Cecchini, X. Jordana, P. Van Hummelen, M. E. Alvarez and L. Holuigue (2009). "Early genomic responses to salicylic acid in Arabidopsis." Plant Mol Biol **70**(1-2): 79-102.

Boller, T. and G. Felix (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.

Boudart, G., E. Jamet, M. Rossignol, C. Lafitte, G. Borderies, A. Jauneau, M. T. Esquerre-Tugaye and R. Pont-Lezica (2005). "Cell wall proteins in apoplastic fluids of Arabidopsis thaliana rosettes: identification by mass spectrometry and bioinformatics." Proteomics **5**(1): 212-221.

Bouwmeester, K., M. de Sain, R. Weide, A. Gouget, S. Klamer, H. Canut and F. Govers (2011). "The lectin receptor kinase LecRK-I.9 is a novel phytophthora resistance component and a potential host target for a RXLR effector." PLoS Pathog **7**(3): e1001327.

Bowling, S. A., A. Guo, H. Cao, A. S. Gordon, D. F. Klessig and X. Dong (1994). "A mutation in Arabidopsis that leads to constitutive expression of systemic acquired resistance." Plant Cell **6**(12): 1845-1857.

Bozkurt, T. O., S. Schornack, M. J. Banfield and S. Kamoun (2012). "Oomycetes, effectors, and all that jazz." Curr Opin Plant Biol **15**(4): 483-492.

Broekaert, W. F., V. A. N. P. J, F. Leyns, H. Joos and W. J. Peumans (1989). "A chitin-binding lectin from stinging nettle rhizomes with antifungal properties." Science **245**(4922): 1100-1102.

Brunner, A., E. M. Keidel, D. Dosch, J. Kellermann and F. Lottspeich (2010). "ICPLQuant - A software for non-isobaric isotopic labeling proteomics." Proteomics **10**(2): 315-326.

Casasoli, M., S. Spadoni, K. S. Lilley, F. Cervone, G. De Lorenzo and B. Mattei (2008). "Identification by 2-D DIGE of apoplastic proteins regulated by oligogalacturonides in Arabidopsis thaliana." Proteomics **8**(5): 1042-1054.

Chanda, B., Y. Xia, M. K. Mandal, K. Yu, K. T. Sekine, Q. M. Gao, D. Selote, Y. Hu, A. Stromberg, D. Navarre, A. Kachroo and P. Kachroo (2011). "Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants." Nat Genet **43**(5): 421-427.

Chapman, S. N. (1998). "Tobamovirus isolation and RNA extraction." Methods Mol Biol **81**: 123-129.

Chaturvedi, R., K. Krothapalli, R. Makandar, A. Nandi, A. A. Sparks, M. R. Roth, R. Welti and J. Shah (2008). "Plastid omega 3-fatty acid desaturase-dependent accumulation of a systemic acquired resistance inducing activity in petiole exudates of Arabidopsis thaliana is independent of jasmonic acid." Plant Journal **54**(1): 106-117.

Chaturvedi, R., B. Venables, R. A. Petros, V. Nalam, M. Y. Li, X. M. Wang, L. J. Takemoto and J. Shah (2012). "An abietane diterpenoid is a potent activator of systemic acquired resistance." Plant Journal **71**(1): 161-172.

Chen, Z., Z. Zheng, J. Huang, Z. Lai and B. Fan (2009). "Biosynthesis of salicylic acid in plants." Plant Signal Behav **4**(6): 493-496.

Chepanoske, C. L., B. E. Richardson, M. von Rechenberg and J. M. Peltier (2005). "Average peptide score: a useful parameter for identification of proteins derived from database searches of liquid chromatography/tandem mass spectrometry data." Rapid Commun Mass Spectrom **19**(1): 9-14.

Chisholm, S. T., G. Coaker, B. Day and B. J. Staskawicz (2006). "Host-microbe interactions: Shaping the evolution of the plant immune response." Cell **124**(4): 803-814.

Cho, J. I., N. Ryoo, J. S. Eom, D. W. Lee, H. B. Kim, S. W. Jeong, Y. H. Lee, Y. K. Kwon, M. H. Cho, S. H. Bhoo, T. R. Hahn, Y. I. Park, I. Hwang, J. Sheen and J. S. Jeon (2009). "Role of the Rice Hexokinases OsHXK5 and OsHXK6 as Glucose Sensors." Plant Physiology **149**(2): 745-759.

Chrispeels, M. J. and N. V. Raikhel (1991). "Lectins, lectin genes, and their role in plant defense." Plant Cell **3**(1): 1-9.

Clarke, S. M., L. A. J. Mur, J. E. Wood and I. M. Scott (2004). "Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in Arabidopsis thaliana." Plant Journal **38**(3): 432-447.

Clough, S. J. and A. F. Bent (1998). "Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana." Plant J **16**(6): 735-743.

Cochrane, F. C., L. B. Davin and N. G. Lewis (2004). "The Arabidopsis phenylalanine ammonia lyase gene family: kinetic characterization of the four PAL isoforms." Phytochemistry **65**(11): 1557-1564.

Cohen, Y., U. Gisi and T. Niderman (1993). "Local and systemic protection against Phytophthora infestans induced in potato and tomato plants by jasmonic acid and jasmonic acid methyl ester." Phytopathology **83**: 1054-1063.

Collier, S. M. and P. Moffett (2009). "NB-LRRs work a "bait and switch" on pathogens." Trends in Plant Science **14**(10): 521-529.

Conrath, U. (2006). "Systemic acquired resistance." Plant Signal Behav **1**(4): 179-184.

Conrath, U. (2009). "Priming of Induced Plant Defense Responses." Plant Innate Immunity **51**: 361-395.

Corthals, G. L., V. C. Wasinger, D. F. Hochstrasser and J. C. Sanchez (2000). "The dynamic range of protein expression: A challenge for proteomic research." Electrophoresis **21**(6): 1104-1115.

Couldridge, C., H. J. Newbury, B. Ford-Lloyd, J. Bale and J. Pritchard (2007). "Exploring plant responses to aphid feeding using a full Arabidopsis microarray reveals a small number of genes with significantly altered expression." Bulletin of Entomological Research **97**(5): 523-532.

Daszkowska-Golec, A. (2011). "Arabidopsis seed germination under abiotic stress as a concert of action of phytohormones." OMICS **15**(11): 763-774.

de Jong, M., B. van Breukelen, F. R. Wittink, F. L. H. Menke, P. J. Weisbeek and G. Van den Ackerveken (2006). "Membrane-associated transcripts in Arabidopsis; their isolation and characterization by DNA microarray analysis and bioinformatics." Plant Journal **46**(4): 708-721.

De Vos, M., W. Van Zaanen, A. Koornneef, J. P. Korzelius, M. Dicke, L. C. Van Loon and C. M. Pieterse (2006). "Herbivore-induced resistance against microbial pathogens in Arabidopsis." Plant Physiol **142**(1): 352-363.

Delaney, T. P., S. Uknes, B. Vernooij, L. Friedrich, K. Weymann, D. Negrotto, T. Gaffney, M. Gut-Rella, H. Kessmann, E. Ward and J. Ryals (1994). "A Central Role of Salicylic Acid in Plant Disease Resistance." Science **266**(5188): 1247-1250.

Delessert, C., K. Kazan, I. W. Wilson, D. Van Der Straeten, J. Manners, E. S. Dennis and R. Dolferus (2005). "The transcription factor ATAF2 represses the expression of pathogenesis-related genes in Arabidopsis." Plant J **43**(5): 745-757.

Dempsey, D. A. and D. F. Klessig (2012). "SOS - too many signals for systemic acquired resistance?" Trends Plant Sci.

Diaz, J., A. ten Have and J. A. van Kan (2002). "The role of ethylene and wound signaling in resistance of tomato to Botrytis cinerea." Plant Physiol **129**(3): 1341-1351.

Doares, S. H., J. Narvaez-Vasquez, A. Conconi and C. A. Ryan (1995). "Salicylic Acid Inhibits Synthesis of Proteinase Inhibitors in Tomato Leaves Induced by Systemin and Jasmonic Acid." Plant Physiol **108**(4): 1741-1746.

Domon, B. and R. Aebersold (2006). "Review - Mass spectrometry and protein analysis." Science **312**(5771): 212-217.

Dunkley, T. P., P. Dupree, R. B. Watson and K. S. Lilley (2004). "The use of isotope-coded affinity tags (ICAT) to study organelle proteomes in Arabidopsis thaliana." Biochem Soc Trans **32**(Pt3): 520-523.

Durrant, W. E. and X. Dong (2004). "Systemic acquired resistance." Annual Review of Phytopathology **42**: 185-209.

Ellis, J. G., G. J. Lawrence and P. N. Dodds (2007). "Further analysis of gene-for-gene disease resistance specificity in flax." Molecular Plant Pathology **8**(1): 103-109.

Elmore, J. M., J. Liu, B. Smith, B. Phinney and G. Coaker (2012). "Quantitative proteomics reveals dynamic changes in the plasma membrane during Arabidopsis immune signaling." Mol Cell Proteomics **11**(4): M111 014555.

Engelsberger, W. R., A. Erban, J. Kopka and W. X. Schulze (2006). "Metabolic labeling of plant cell cultures with K(15)NO3 as a tool for quantitative analysis of proteins and metabolites." Plant Methods **2**: 14.

Erb, M., S. Meldau and G. A. Howe (2012). "Role of phytohormones in insect-specific plant reactions." Trends in Plant Science **17**(5): 250-259.

Fabro, G., J. Steinbrenner, M. Coates, N. Ishaque, L. Baxter, D. J. Studholme, E. Korner, R. L. Allen, S. J. Piquerez, A. Rougon-Cardoso, D. Greenshields, R. Lei, J. L. Badel, M. C. Caillaud, K. H. Sohn, G. Van den Ackerveken, J. E. Parker, J. Beynon and J. D. Jones (2011). "Multiple candidate effectors from the oomycete pathogen Hyaloperonospora arabidopsidis suppress host plant immunity." PLoS Pathog **7**(11): e1002348.

Feys, B. J., L. J. Moisan, M. A. Newman and J. E. Parker (2001). "Direct interaction between the Arabidopsis disease resistance signaling proteins, EDS1 and PAD4." EMBO J **20**(19): 5400-5411.

Feys, B. J., M. Wiermer, R. A. Bhat, L. J. Moisan, N. Medina-Escobar, C. Neu, A. Cabral and J. E. Parker (2005). "Arabidopsis SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity." Plant Cell **17**(9): 2601-2613.

Fields, S. and O. Song (1989). "A novel genetic system to detect protein-protein interactions." Nature **340**(6230): 245-246.

Fischer, R., C. Vaquero-Martin, M. Sack, J. Drossard, N. Emans and U. Commandeur (1999). "Towards molecular farming in the future: transient protein expression in plants." Biotechnol Appl Biochem **30 (Pt 2)**: 113-116.

Friedrich, L., K. Lawton, W. Ruess, P. Masner, N. Specker, M. G. Rella, B. Meier, S. Dincher, T. Staub, S. Uknes, J. P. Metraux, H. Kessmann and J. Ryals (1996). "A benzothiadiazole derivative induces systemic acquired resistance in tobacco." Plant Journal **10**(1): 61-70.

Frohlich, A., F. Gaupels, H. Sarioglu, C. Holzmeister, M. Spannagl, J. Durner and C. Lindermayr (2012). "Looking Deep Inside: Detection of Low-Abundance Proteins in Leaf Extracts of Arabidopsis and Phloem Exudates of Pumpkin." Plant Physiology **159**(3): 902-914.

Fu, Z. Q., M. Guo, B. R. Jeong, F. Tian, T. E. Elthon, R. L. Cerny, D. Staiger and J. R. Alfano (2007). "A type III effector ADP-ribosylates RNA-binding proteins and quells plant immunity." Nature **447**(7142): 284-U281.

Gaffney, T., L. Friedrich, B. Vernooij, D. Negrotto, G. Nye, S. Uknes, E. Ward, H. Kessmann and J. Ryals (1993). "Requirement of salicylic Acid for the induction of systemic acquired resistance." Science **261**(5122): 754-756.

Galan, J. E. and A. Collmer (1999). "Type III secretion machines: Bacterial devices for protein delivery into host cells." Science **284**(5418): 1322-1328.

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

Gaupels, F., H. Sarioglu, M. Beckmann, B. Hause, M. Spannagl, J. Draper, C. Lindermayr and J. Durner (2012). "Deciphering systemic wound responses of the pumpkin extrafascicular phloem by metabolomics and stable isotope-coded protein labeling (ICPL)." Plant Physiol.

Gibson, D. M., S. Stack, K. Krell and J. House (1982). "A Comparison of Soybean Agglutinin in Cultivars Resistant and Susceptible to Phytophthora megasperma var. sojae (Race 1)." Plant Physiol **70**(2): 560-566.

Gilardoni, P. A., C. Hettenhausen, I. T. Baldwin and G. Bonaventure (2011). "Nicotiana attenuata LECTIN RECEPTOR KINASE1 suppresses the insect-mediated inhibition of induced defense responses during Manduca sexta herbivory." Plant Cell **23**(9): 3512-3532.

Glazebrook, J. (2005). "Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens." Annual Review of Phytopathology **43**: 205-227.

Gohre, V. and S. Robatzek (2008). "Breaking the barriers: microbial effector molecules subvert plant immunity." Annu Rev Phytopathol **46**: 189-215.

Goldstein, I. J. and C. E. Hayes (1978). "The lectins: carbohydrate-binding proteins of plants and animals." Adv Carbohydr Chem Biochem **35**: 127-340.

Gomez-Gomez, L. and T. Boller (2000). "FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis." Molecular Cell **5**(6): 1003-1011.

Grant, M. R. and J. D. Jones (2009). "Hormone (dis)harmony moulds plant health and disease." Science **324**(5928): 750-752.

Grbic, V. and A. B. Bleecker (1995). "Ethylene regulates the timing of leaf senescence in Arabidopsis." Plant Journal **v. 8(4)**: p. 595-602.

Greeff, C., M. Roux, J. Mundy and M. Petersen (2012). "Receptor-like kinase complexes in plant innate immunity." Front Plant Sci **3**: 209.

Gruhler, A., W. X. Schulze, R. Matthiesen, M. Mann and O. N. Jensen (2005). "Stable isotope labeling of Arabidopsis thaliana cells and quantitative proteomics by mass spectrometry." Mol Cell Proteomics **4**(11): 1697-1709.

Guelette, B. S., U. F. Benning and S. Hoffmann-Benning (2012). "Identification of lipids and lipid-binding proteins in phloem exudates from Arabidopsis thaliana." J Exp Bot **63**(10): 3603-3616.

Gupta, V., M. G. Willits and J. Glazebrook (2000). "Arabidopsis thaliana EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: Evidence for inhibition of jasmonic acid signaling by SA." Molecular Plant-Microbe Interactions **13**(5): 503-511.

Gygi, S. P., B. Rist, S. A. Gerber, F. Turecek, M. H. Gelb and R. Aebersold (1999). "Quantitative analysis of complex protein mixtures using isotope-coded affinity tags." Nat Biotechnol **17**(10): 994-999.

Hamblin, J. and S. P. Kent (1973). "Possible role of phytohaemagglutinin in Phaseolus vulgaris L." Nat New Biol **245**(140): 28-30.

HammondKosack, K. E. and J. D. G. Jones (1997). "Plant disease resistance genes." Annual Review of Plant Physiology and Plant Molecular Biology **48**: 575-607.

Hannon, G. J. (2002). "RNA interference." Nature **418**(6894): 244-251.

Hayashi, F., K. D. Smith, A. Ozinsky, T. R. Hawn, E. C. Yi, D. R. Goodlett, J. K. Eng, S. Akira, D. M. Underhill and A. Aderem (2001). "The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5." Nature **410**(6832): 1099-1103.

He, Y. H., H. Fukushige, D. F. Hildebrand and S. S. Gan (2002). "Evidence supporting a role of jasmonic acid in Arabidopsis leaf senescence." Plant Physiology **128**(3): 876-884.

Heidrich, K., L. Wirthmueller, C. Tasset, C. Pouzet, L. Deslandes and J. E. Parker (2011). "Arabidopsis EDS1 connects pathogen effector recognition to cell compartment-specific immune responses." Science **334**(6061): 1401-1404.

Herbers, K., P. Meuwly, W. B. Frommer, J. P. Metraux and U. Sonnewald (1996). "Systemic Acquired Resistance Mediated by the Ectopic Expression of Invertase: Possible Hexose Sensing in the Secretory Pathway." Plant Cell **8**(5): 793-803.

Herbers, K., P. Meuwly, J. P. Metraux and U. Sonnewald (1996). "Salicylic acid-independent induction of pathogenesis-related protein transcripts by sugars is dependent on leaf developmental stage." FEBS Lett **397**(2-3): 239-244.

Hewezi, T., P. Howe, T. R. Maier, R. S. Hussey, M. G. Mitchum, E. L. Davis and T. J. Baum (2008). "Cellulose binding protein from the parasitic nematode Heterodera schachtii interacts with Arabidopsis pectin methylesterase: cooperative cell wall modification during parasitism." Plant Cell **20**(11): 3080-3093.

Hillung, J., J. M. Cuevas and S. F. Elena (2012). "Transcript Profiling of Different Arabidopsis thaliana Ecotypes in Response to Tobacco etch potyvirus Infection." Front Microbiol **3**: 229.

Hofius, D., T. Schultz-Larsen, J. Joensen, D. I. Tsitsigiannis, N. H. Petersen, O. Mattsson, L. B. Jorgensen, J. D. Jones, J. Mundy and M. Petersen (2009). "Autophagic components contribute to hypersensitive cell death in Arabidopsis." Cell **137**(4): 773-783.

Hu, Q., R. J. Noll, H. Li, A. Makarov, M. Hardman and R. Graham Cooks (2005). "The Orbitrap: a new mass spectrometer." J Mass Spectrom **40**(4): 430-443.

Hueck, C. J. (1998). "Type III protein secretion systems in bacterial pathogens of animals and plants." Microbiology and Molecular Biology Reviews **62**(2): 379-+.

Ishizaki, T., C. Ohsumi, K. Totsuka and D. Igarashi (2009). "Analysis of glutamate homeostasis by overexpression of Fd-GOGAT gene in Arabidopsis thaliana." Amino Acids **38**(3): 943-950.

Jakab, G., V. Cottier, V. Toquin, G. Rigoli, L. Zimmerli, J. P. Metraux and B. Mauch-Mani (2001). "beta-Aminobutyric acid-induced resistance in plants." European Journal of Plant Pathology **107**(1): 29-37.

Jamai, A., P. A. Salome, S. H. Schilling, A. P. Weber and C. R. McClung (2009). "Arabidopsis photorespiratory serine hydroxymethyltransferase activity requires the mitochondrial accumulation of ferredoxin-dependent glutamate synthase." Plant Cell **21**(2): 595-606.

Janeway, C. A., Jr. and R. Medzhitov (2002). "Innate immune recognition." Annu Rev Immunol **20**: 197-216.

Jang, J. C., P. Leon, L. Zhou and J. Sheen (1997). "Hexokinase as a sugar sensor in higher plants." Plant Cell **9**(1): 5-19.

Jaskiewicz, M., U. Conrath and C. Peterhansel (2011). "Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response." Embo Reports **12**(1): 50-55.

Jefferson, R. A. (1989). "The GUS reporter gene system." Nature **342**(6251): 837-838.

Jirage, D., T. L. Tootle, T. L. Reuber, L. N. Frost, B. J. Feys, J. E. Parker, F. M. Ausubel and J. Glazebrook (1999). "Arabidopsis thaliana PAD4 encodes a lipase-like gene that is important for salicylic acid signaling." Proceedings of the National Academy of Sciences of the United States of America **96**(23): 13583-13588.

Jones, A. M., M. H. Bennett, J. W. Mansfield and M. Grant (2006). "Analysis of the defence phosphoproteome of Arabidopsis thaliana using differential mass tagging." Proteomics **6**(14): 4155-4165.

Jones, A. M. E., V. Thomas, M. H. Bennett, J. Mansfield and M. Grant (2006). "Modifications to the arabidopsis defense proteome occur prior to significant transcriptional change in response to inoculation with Pseudomonas syringae." Plant Physiology **142**(4): 1603-1620.

Jones, J. D. G. and J. L. Dangl (2006). "The plant immune system." Nature **444**(7117): 323-329.

Jung, C., S. H. Lyou, S. Yeu, M. A. Kim, S. Rhee, M. Kim, J. S. Lee, Y. D. Choi and J. J. Cheong (2007). "Microarray-based screening of jasmonate-responsive genes in Arabidopsis thaliana." Plant Cell Rep **26**(7): 1053-1063.

Jung, C., S. Y. Yeu, Y. J. Koo, M. Kim, Y. Do Choi and J. J. Cheong (2007). "Transcript profile of transgenic Arabidopsis constitutively producing methyl jasmonate." Journal of Plant Biology **50**(1): 12-17.

Jung, H. W., T. J. Tschaplinski, L. Wang, J. Glazebrook and J. T. Greenberg (2009). "Priming in systemic plant immunity." Science **324**(5923): 89-91.

Kaffarnik, F. A., A. M. Jones, J. P. Rathjen and S. C. Peck (2009). "Effector proteins of the bacterial pathogen Pseudomonas syringae alter the extracellular proteome of the host plant, Arabidopsis thaliana." Mol Cell Proteomics **8**(1): 145-156.

Kallenbach, M., I. T. Baldwin and G. Bonaventure (2009). "A rapid and sensitive method for the simultaneous analysis of aliphatic and polar molecules containing free carboxyl groups in plant extracts by LC-MS/MS." Plant Methods **5**: 17.

Keller, A., A. I. Nesvizhskii, E. Kolker and R. Aebersold (2002). "Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search." Analytical Chemistry **74**(20): 5383-5392.

Kellermann, J. (2008). "ICPL--isotope-coded protein label." Methods Mol Biol **424**: 113-123.

Kende, H. and J. A. D. Zeevaart (1997). "The five ''classical'' plant hormones." Plant Cell **9**(7): 1197-1210.

Kessmann, H., T. Staub, C. Hofmann, T. Maetzke, J. Herzog, E. Ward, S. Uknes and J. Ryals (1994). "Induction of systemic acquired disease resistance in plants by chemicals." Annu Rev Phytopathol **32**: 439-459.

Kissen, R., P. Winge, D. H. Tran, T. S. Jorstad, T. R. Storseth, T. Christensen and A. M. Bones (2010). "Transcriptional profiling of an Fd-GOGAT1/GLU1 mutant in Arabidopsis thaliana reveals a multiple stress response and extensive reprogramming of the transcriptome." Bmc Genomics **11**: 190.

Kloek, A. P., M. L. Verbsky, S. B. Sharma, J. E. Schoelz, J. Vogel, D. F. Klessig and B. N. Kunkel (2001). "Resistance to Pseudomonas syringae conferred by an Arabidopsis thaliana coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms." Plant Journal **26**(5): 509-522.

Koornneef, A., A. Leon-Reyes, T. Ritsema, A. Verhage, F. C. Den Otter, L. C. Van Loon and C. M. J. Pieterse (2008). "Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation." Plant Physiology **147**(3): 1358-1368.

Koornneef, A. and C. M. J. Pieterse (2008). "Cross talk in defense signaling." Plant Physiology **146**(3): 839-844.

Koornneef, M., L. Bentsink and H. Hilhorst (2002). "Seed dormancy and germination." Curr Opin Plant Biol **5**(1): 33-36.

Krinke, O., E. Ruelland, O. Valentova, C. Vergnolle, J. P. Renou, L. Taconnat, M. Flemr, L. Burketova and A. Zachowski (2007). "Phosphatidylinositol 4-kinase activation is an early response to salicylic acid in Arabidopsis suspension cells." Plant Physiol **144**(3): 1347-1359.

Kusnierczyk, A., P. Winge, T. S. Jorstad, J. Troczynska, J. T. Rossiter and A. M. Bones (2008). "Towards global understanding of plant defence against aphids - timing and dynamics of early Arabidopsis defence responses to cabbage aphid (Brevicoryne brassicae) attack." Plant Cell and Environment **31**(8): 1097-1115.

Kwon, H. K., R. Yokoyama and K. Nishitani (2005). "A proteomic approach to apoplastic proteins involved in cell wall regeneration in protoplasts of Arabidopsis suspension-cultured cells." Plant Cell Physiol **46**(6): 843-857.

Laemmli, U. K. (1970). "Cleavage of structural proteins during the assembly of the head of bacteriophage T4." Nature **227**(5259): 680-685.

Lara-Tejero, M., J. Kato, S. Wagner, X. Y. Liu and J. E. Galan (2011). "A Sorting Platform Determines the Order of Protein Secretion in Bacterial Type III Systems." Science **331**(6021): 1188-1191.

Lascombe, M. B., B. Bakan, N. Buhot, D. Marion, J. P. Blein, V. Larue, C. Lamb and T. Prange (2008). "The structure of "defective in induced resistance" protein of Arabidopsis thaliana, DIR1, reveals a new type of lipid transfer protein." Protein Sci **17**(9): 1522-1530.

Lawton, K. A., S. L. Potter, S. Uknes and J. Ryals (1994). "Acquired Resistance Signal Transduction in Arabidopsis Is Ethylene Independent." Plant Cell **6**(5): 581-588.

Lee, J., T. M. Bricker, M. Lefevre, S. R. M. Pinson and J. H. Oard (2006). "Proteomic and genetic approaches to identifying defence-related proteins in rice challenged with the fungal pathogen Rhizoctonia solani." Molecular Plant Pathology **7**(5): 405-416.

Lelievre, J. M., A. Latche, B. Jones, M. Bouzayen and J. C. Pech (1997). "Ethylene and fruit ripening." Physiologia Plantarum **101**(4): 727-739.

Leon-Reyes, A., Y. J. Du, A. Koornneef, S. Proietti, A. P. Korbes, J. Memelink, C. M. J. Pieterse and T. Ritsema (2010). "Ethylene Signaling Renders the Jasmonate Response of Arabidopsis Insensitive to Future Suppression by Salicylic Acid." Molecular Plant-Microbe Interactions **23**(2): 187-197.

Leonhardt, N., J. M. Kwak, N. Robert, D. Waner, G. Leonhardt and J. I. Schroeder (2004). "Microarray expression analyses of Arabidopsis guard cells and isolation of a recessive abscisic acid hypersensitive protein phosphatase 2C mutant." Plant Cell **16**(3): 596-615.

Lerner, D. R. and N. V. Raikhel (1992). "The gene for stinging nettle lectin (Urtica dioica agglutinin) encodes both a lectin and a chitinase." J Biol Chem **267**(31): 22694.

Lewsey, M. G., A. M. Murphy, D. MacLean, N. Dalchau, J. H. Westwood, K. Macaulay, M. H. Bennett, M. Moulin, D. E. Hanke, G. Powell, A. G. Smith and J. P. Carr (2010). "Disruption of Two Defensive Signaling Pathways by a Viral RNA Silencing Suppressor." Molecular Plant-Microbe Interactions **23**(7): 835-845.

Li, L., C. Y. Li, G. I. Lee and G. A. Howe (2002). "Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato." Proceedings of the National Academy of Sciences of the United States of America **99**(9): 6416-6421.

Ling, H. (2008). "Sequence analysis of GDSL lipase gene family in Arabidopsis thaliana." Pak J Biol Sci **11**(5): 763-767.

Liu, P. P., C. C. von Dahl and D. F. Klessig (2011). "The extent to which methyl salicylate is required for signaling systemic acquired resistance is dependent on exposure to light after infection." Plant Physiol.

Liu, P. P., C. C. von Dahl, S. W. Park and D. F. Klessig (2011). "Interconnection between methyl salicylate and lipid-based long-distance signaling during the development of systemic acquired resistance in Arabidopsis and tobacco." Plant Physiol **155**(4): 1762-1768.

Liu, P. P., Y. Yang, E. Pichersky and D. F. Klessig (2010). "Altering expression of benzoic acid/salicylic acid carboxyl methyltransferase 1 compromises systemic acquired resistance and PAMP-triggered immunity in arabidopsis." Mol Plant Microbe Interact **23**(1): 82-90.

Logemann, J., J. Schell and L. Willmitzer (1987). "Improved method for the isolation of RNA from plant tissues." Anal Biochem **163**(1): 16-20.

Lorenzo, O., R. Piqueras, J. J. Sanchez-Serrano and R. Solano (2003). "ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense." Plant Cell **15**(1): 165-178.

Loris, R., T. Hamelryck, J. Bouckaert and L. Wyns (1998). "Legume lectin structure." Biochim Biophys Acta **1383**(1): 9-36.

Lottspeich, F., A. Schmidt and J. Kellermann (2004). "ICPL - A new labeling strategy for multiplexed quantitative proteomics." Molecular & Cellular Proteomics **3**(10): S240-S240.

Luna, E., T. J. A. Bruce, M. R. Roberts, V. Flors and J. Ton (2012). "Next-Generation Systemic Acquired Resistance." Plant Physiology **158**(2): 844-853.

Lyou, S. H., H. J. Park, C. Jung, H. B. Sohn, G. Lee, C. H. Kim, M. Kim, Y. D. Choi and J. J. Cheong (2009). "The Arabidopsis AtLEC gene encoding a lectin-like protein is up-regulated by multiple stimuli including developmental signal, wounding, jasmonate, ethylene, and chitin elicitor." Mol Cells **27**(1): 75-81.

Machuka, J., E. J. M. Van Damme, W. J. Peumans and L. E. N. Jackai (1999). "Effect of plant lectins on larval development of the legume pod borer, Maruca vitrata." Entomologia Experimentalis Et Applicata **93**(2): 179-187.

Mackey, D., B. F. Holt, A. Wiig and J. L. Dangl (2002). "RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis." Cell **108**(6): 743-754.

Mackey, D. and A. J. McFall (2006). "MAMPs and MIMPs: proposed classifications for inducers of innate immunity." Molecular Microbiology **61**(6): 1365-1371.

Makarov, A. (2000). "Electrostatic axially harmonic orbital trapping: a high-performance technique of mass analysis." Anal Chem **72**(6): 1156-1162.

Maldonado, A. M., P. Doerner, R. A. Dixon, C. J. Lamb and R. K. Cameron (2002). "A putative lipid transfer protein involved in systemic resistance signalling in Arabidopsis." Nature **419**(6905): 399-403.

Mallick, P. and B. Kuster (2010). "Proteomics: a pragmatic perspective." Nature Biotechnology **28**(7): 695-709.

Mandal, M. K., B. Chanda, Y. Xia, K. Yu, K. Sekine, Q. M. Gao, D. Selote, A. Kachroo and P. Kachroo (2011). "Glycerol-3-phosphate and systemic immunity." Plant Signal Behav **6**(11).

Manthe, B., M. Schulz and H. Schnabl (1992). "Effects of salicylic acid on growth and stomatal movements ofVicia faba L.: Evidence for salicylic acid metabolization." Journal of Chemical Ecology **Volume 18**(Number 9): 1525-1539.

Martin, G. B., A. J. Bogdanove and G. Sessa (2003). "Understanding the functions of plant disease resistance proteins." Annu Rev Plant Biol **54**: 23-61.

MauchMani, B. and A. J. Slusarenko (1996). "Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of arabidopsis to Peronospora parasitica." Plant Cell **8**(2): 203-212.

McConn, M., R. A. Creelman, E. Bell, J. E. Mullet and J. Browse (1997). "Jasmonate is essential for insect defense Arabidopsis." Proceedings of the National Academy of Sciences of the United States of America **94**(10): 5473-5477.

Melander, M., I. Ahman, I. Kamnert and A. C. Stromdahl (2003). "Pea lectin expressed transgenically in oilseed rape reduces growth rate of pollen beetle larvae." Transgenic Res **12**(5): 555-567.

Metraux, J. P. (2002). "Recent breakthroughs in the study of salicylic acid biosynthesis." Trends Plant Sci **7**(8): 332-334.

Meuwly, P., W. Molders, A. Buchala and J. P. Metraux (1995). "Local and Systemic Biosynthesis of Salicylic Acid in Infected Cucumber Plants." Plant Physiol **109**(3): 1107-1114.

Meyers, B. C., A. W. Dickerman, R. W. Michelmore, S. Sivaramakrishnan, B. W. Sobral and N. D. Young (1999). "Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily." Plant Journal **20**(3): 317-332.

Miao, Y., T. Laun, P. Zimmermann and U. Zentgraf (2004). "Targets of the WRKY53 transcription factor and its role during leaf senescence in Arabidopsis." Plant Molecular Biology **55**(6): 853-867.

Miao, Y. S. and L. W. Jiang (2007). "Transient expression of fluorescent fusion proteins in protoplasts of suspension cultured cells." Nature Protocols **2**(10): 2348-2353.

Minic, Z., C. Rihouey, C. T. Do, P. Lerouge and L. Jouanin (2004). "Purification and characterization of enzymes exhibiting beta-D-xylosidase activities in stem tissues of Arabidopsis." Plant Physiology **135**(2): 867-878.

Moore, B., L. Zhou, F. Rolland, Q. Hall, W. H. Cheng, Y. X. Liu, I. Hwang, T. Jones and J. Sheen (2003). "Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling." Science **300**(5617): 332-336.

Mur, L. A., P. Kenton, R. Atzorn, O. Miersch and C. Wasternack (2006). "The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death." Plant Physiol **140**(1): 249-262.

Mur, L. A., P. Kenton, A. J. Lloyd, H. Ougham and E. Prats (2008). "The hypersensitive response; the centenary is upon us but how much do we know?" J Exp Bot **59**(3): 501-520.

Narusaka, Y., M. Narusaka, T. Horio and H. Ishii (1999). "Comparison of local and systemic induction of acquired disease resistance in cucumber plants treated with benzothiadiazoles or salicylic acid." Plant Cell Physiol **40**(4): 388-395.

Nicaise, V., M. Roux and C. Zipfel (2009). "Recent advances in PAMP-triggered immunity against bacteria: pattern recognition receptors watch over and raise the alarm." Plant Physiol **150**(4): 1638-1647.

Niki, T., I. Mitsuhara, S. Seo, N. Ohtsubo and Y. Ohashi (1998). "Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves." Plant and Cell Physiology **39**(5): 500-507.

Nishimura, M. T. and J. L. Dangl (2010). "Arabidopsis and the plant immune system." Plant J **61**(6): 1053-1066.

Nogueira, F. C., G. Palmisano, V. Schwammle, F. A. Campos, M. R. Larsen, G. B. Domont and P. Roepstorff (2012). "Performance of isobaric and isotopic labeling in quantitative plant proteomics." J Proteome Res **11**(5): 3046-3052.

O'Donnell, P. J., J. B. Jones, F. R. Antoine, J. Ciardi and H. J. Klee (2001). "Ethylene-dependent salicylic acid regulates an expanded cell death response to a plant pathogen." Plant Journal **25**(3): 315-323.

Oeljeklaus, S., H. E. Meyer and B. Warscheid (2009). "Advancements in plant proteomics using quantitative mass spectrometry." J Proteomics **72**(3): 545-554.

Oh, I. S., A. R. Park, M. S. Bae, S. J. Kwon, Y. S. Kim, J. E. Lee, N. Y. Kang, S. Lee, H. Cheong and O. K. Park (2005). "Secretome analysis reveals an Arabidopsis lipase involved in defense against Alternaria brassicicola." Plant Cell **17**(10): 2832-2847.

Ong, S. E., B. Blagoev, I. Kratchmarova, D. B. Kristensen, H. Steen, A. Pandey and M. Mann (2002). "Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics." Mol Cell Proteomics **1**(5): 376-386.

Pallas, J. A., N. L. Paiva, C. Lamb and R. A. Dixon (1996). "Tobacco plants epigenetically suppressed in phenylalanine ammonia-lyase expression do not develop systemic acquired resistance in response to infection by tobacco mosaic virus." Plant Journal **10**(2): 281-293.

Paradela, A., M. Marcilla, R. Navajas, L. Ferreira, A. Ramos-Fernandez, M. Fernandez, J. F. Mariscotti, F. Garcia-del Portillo and J. P. Albar (2010). "Evaluation of isotope-coded protein labeling (ICPL) in the quantitative analysis of complex proteomes." Talanta **80**(4): 1496-1502.

Park, S. W., E. Kaimoyo, D. Kumar, S. Mosher and D. F. Klessig (2007). "Methyl salicylate is a critical mobile signal for plant systemic acquired resistance." Science **318**(5847): 113-116.

Park, S. W., P. P. Liu, F. Forouhar, A. C. Vlot, L. Tong, K. Tietjen and D. F. Klessig (2009). "Use of a Synthetic Salicylic Acid Analog to Investigate the Roles of Methyl Salicylate and Its Esterases in Plant Disease Resistance." Journal of Biological Chemistry **284**(11): 7307-7317.

Parker, J. E. (2003). "Plant recognition of microbial patterns." Trends Plant Sci **8**(6): 245-247.

Parker, J. E., E. B. Holub, L. N. Frost, A. Falk, N. D. Gunn and M. J. Daniels (1996). "Characterization of eds1, a mutation in Arabidopsis suppressing resistance to Peronospora parasitica specified by several different RPP genes." Plant Cell **8**(11): 2033-2046.

Patterson, S. E. and A. B. Bleecker (2004). "Ethylene-dependent and -independent processes associated with floral organ abscission in Arabidopsis." Plant Physiology **134**(1): 194-203.

Pellegrini, L., O. Rohfritsch, B. Fritig and M. Legrand (1994). "Phenylalanine ammonia-lyase in tobacco. Molecular cloning and gene expression during the hypersensitive reaction to tobacco mosaic virus and the response to a fungal elicitor." Plant Physiol **106**(3): 877-886.

Peng, D. H., D. W. Qiu, L. F. Ruan, C. F. Zhou and M. Sun (2011). "Protein elicitor PemG1 from Magnaporthe grisea induces systemic acquired resistance (SAR) in plants." Mol Plant Microbe Interact **24**(10): 1239-1246.

Penninckx, I. A. M. A., B. P. H. J. Thomma, A. Buchala, J. P. Metraux and W. F. Broekaert (1998). "Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis." Plant Cell **10**(12): 2103-2113.

Perez-Bueno, M. L., J. Rahoutei, C. Sajnani, I. Garcia-Luque and M. Baron (2004). "Proteomic analysis of the oxygen-evolving complex of photosystem II under biotec stress: Studies on Nicotiana benthamiana infected with tobamoviruses." Proteomics **4**(2): 418-425.

Petricka, J. J., C. M. Winter and P. N. Benfey (2012). "Control of Arabidopsis root development." Annu Rev Plant Biol **63**: 563-590.

Peumans, W. J. and E. J. Van Damme (1995). "Lectins as plant defense proteins." Plant Physiol **109**(2): 347-352.

Pieterse, C. M. J., A. Leon-Reyes, S. Van der Ent and S. C. M. Van Wees (2009). "Networking by small-molecule hormones in plant immunity." Nature Chemical Biology **5**(5): 308-316.

Powell, K. S. (2001). "Antimetabolic effects of plant lectins towards nymphal stages of the planthoppers Tarophagous proserpina and Nilaparvata lugens." Entomologia Experimentalis Et Applicata **99**(1): 71-77.

Prasad, B. D., G. Creissen, C. Lamb and B. B. Chattoo (2009). "Overexpression of rice (Oryza sativa L.) OsCDR1 leads to constitutive activation of defense responses in rice and Arabidopsis." Mol Plant Microbe Interact **22**(12): 1635-1644.

Prasad, B. D., G. Creissen, C. Lamb and B. B. Chattoo (2010). "Heterologous expression and characterization of recombinant OsCDR1, a rice aspartic proteinase involved in disease resistance." Protein Expr Purif.

Pre, M., M. Atallah, A. Champion, M. De Vos, C. M. J. Pieterse and J. Memelink (2008). "The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense." Plant Physiology **147**(3): 1347-1357.

Pruss, G. J., E. W. Nester and V. Vance (2008). "Infiltration with Agrobacterium tumefaciens induces host defense and development-dependent responses in the infiltrated zone." Mol Plant Microbe Interact **21**(12): 1528-1538.

Pusztai, A., S. W. Ewen, G. Grant, D. S. Brown, J. C. Stewart, W. J. Peumans, E. J. Van Damme and S. Bardocz (1993). "Antinutritive effects of wheat-germ agglutinin and other N-acetylglucosamine-specific lectins." Br J Nutr **70**(1): 313-321.

Rajjou, L., M. Belghazi, R. Huguet, C. Robin, A. Moreau, C. Job and D. Job (2006). "Proteomic investigation of the effect of salicylic acid on Arabidopsis seed germination and establishment of early defense mechanisms." Plant Physiology **141**(3): 910-923.

Rappsilber, J., M. Mann and Y. Ishihama (2007). "Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips." Nat Protoc **2**(8): 1896-1906.

Raskin, I. (1992). "Role of Salicylic Acid in Plants." Annual Review of Plant Physiology and Plant Molecular Biology **Vol. 43**: 439-463.

Rate, D. N., J. V. Cuenca, G. R. Bowman, D. S. Guttman and J. T. Greenberg (1999). "The gain-of-function Arabidopsis acd6 mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth." Plant Cell **11**(9): 1695-1708.

Reid, M. S. and M.-J. Wu (1992). "Ethylene and flower senescence." Plant Growth Regulation **Volume 11, Number 1**: 37-43.

Reymond, P., H. Weber, M. Damond and E. E. Farmer (2000). "Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis." Plant Cell **12**(5): 707-719.

Rietz, S., A. Stamm, S. Malonek, S. Wagner, D. Becker, N. Medina-Escobar, A. C. Vlot, B. J. Feys, K. Niefind and J. E. Parker (2011). "Different roles of Enhanced Disease Susceptibility1 (EDS1) bound to and dissociated from Phytoalexin Deficient4 (PAD4) in Arabidopsis immunity." New Phytol **191**(1): 107-119.

Robert-Seilaniantz, A., L. Navarro, R. Bari and J. D. Jones (2007). "Pathological hormone imbalances." Current Opinion in Plant Biology **10**(4): 372-379.

Roberts, D. D. and I. J. Goldstein (1983). "Adenine binding sites of the lectin from lima beans (Phaseolus lunatus)." J Biol Chem **258**(22): 13820-13824.

Rolland, F., E. Baena-Gonzalez and J. Sheen (2006). "Sugar sensing and signaling in plants: Conserved and novel mechanisms." Annual Review of Plant Biology **57**: 675-709.

Rosebrock, T. R., L. Zeng, J. J. Brady, R. B. Abramovitch, F. Xiao and G. B. Martin (2007). "A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt plant immunity." Nature **448**(7151): 370-374.

Ross, A. F. (1961). "Systemic acquired resistance induced by localized virus infections in plants." Virology **14**: 340-358.

Ross, P. L., Y. N. Huang, J. N. Marchese, B. Williamson, K. Parker, S. Hattan, N. Khainovski, S. Pillai, S. Dey, S. Daniels, S. Purkayastha, P. Juhasz, S. Martin, M. Bartlet-Jones, F. He, A. Jacobson and D. J. Pappin (2004).

"Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents." Mol Cell Proteomics **3**(12): 1154-1169.

Rossel, J. B., I. W. Wilson and B. J. Pogson (2002). "Global changes in gene expression in response to high light in Arabidopsis." Plant Physiology **130**(3): 1109-1120.

Ruzvidzo, O., L. Donaldson, A. Valentine and C. Gehring (2011). "The Arabidopsis thaliana natriuretic peptide AtPNP-A is a systemic regulator of leaf dark respiration and signals via the phloem." J Plant Physiol **168**(14): 1710-1714.

Ryals, J., S. Uknes and E. Ward (1994). "Systemic Acquired Resistance." Plant Physiol **104**(4): 1109-1112.

Ryals, J. A., U. H. Neuenschwander, M. G. Willits, A. Molina, H. Y. Steiner and M. D. Hunt (1996). "Systemic acquired resistance." Plant Cell **8**(10): 1809-1819.

Sadeghi, A., G. Smagghe, S. Broeders, J. P. Hernalsteens, H. De Greve, W. J. Peumans and E. J. Van Damme (2008). "Ectopically expressed leaf and bulb lectins from garlic (Allium sativum L.) protect transgenic tobacco plants against cotton leafworm (Spodoptera littoralis)." Transgenic Res **17**(1): 9-18.

Sarioglu, H., S. Brandner, C. Jacobsen, T. Meindl, A. Schmidt, J. Kellermann, F. Lottspeich and U. Andrae (2006). "Quantitative analysis of 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced proteome alterations in 5L rat hepatoma cells using isotope-coded protein labels." Proteomics **6**(8): 2407-2421.

Schenk, P. M., K. Kazan, I. Wilson, J. P. Anderson, T. Richmond, S. C. Somerville and J. M. Manners (2000). "Coordinated plant defense responses in Arabidopsis revealed by microarray analysis." Proceedings of the National Academy of Sciences of the United States of America **97**(21): 11655-11660.

Schmidt, A. (2005). "A novel strategy for quantitative proteomics using isotope-coded protein labels (vol 5, pg 4, 2005)." Proteomics **5**(3): 826-826.

Schwessinger, B. and C. Zipfel (2008). "News from the frontline: recent insights into PAMP-triggered immunity in plants." Curr Opin Plant Biol **11**(4): 389-395.

Shah, J. (2009). "Plants under attack: systemic signals in defence." Current Opinion in Plant Biology **12**(4): 459-464.

Shen, Q. H., F. Zhou, S. Bieri, T. Haizel, K. Shirasu and P. Schulze-Lefert (2003). "Recognition specificity and RAR1/SGT1 dependence in barley Mla disease resistance genes to the powdery mildew fungus." Plant Cell **15**(3): 732-744.

Shimomura, O., F. H. Johnson and Y. Saiga (1962). "Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea." J Cell Comp Physiol **59**: 223-239.

Shiu, S. H. and A. B. Bleecker (2001). "Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases." Proc Natl Acad Sci U S A **98**(19): 10763-10768.

Simoes, I., R. Faro, D. Bur and C. Faro (2007). "Characterization of recombinant CDR1, an Arabidopsis aspartic proteinase involved in disease resistance." J Biol Chem **282**(43): 31358-31365.

Spoel, S. H. and X. Dong (2012). "How do plants achieve immunity? Defence without specialized immune cells." Nat Rev Immunol **12**(2): 89-100.

Spoel, S. H. and X. N. Dong (2008). "Making sense of hormone crosstalk during plant immune responses." Cell Host & Microbe **3**(6): 348-351.

Stacey, G., C. B. McAlvin, S. Y. Kim, J. Olivares and M. J. Soto (2006). "Effects of endogenous salicylic acid on nodulation in the model legumes Lotus japonicus and Medicago truncatula." Plant Physiology **141**(4): 1473-1481.

Staswick, P. E. (2008). "JAZing up jasmonate signaling." Trends Plant Sci **13**(2): 66-71.

Stintzi, A., T. Heitz, V. Prasad, S. Wiedemann-Merdinoglu, S. Kauffmann, P. Geoffroy, M. Legrand and B. Fritig (1993). "Plant 'pathogenesis-related' proteins and their role in defense against pathogens." Biochimie **75**(8): 687-706.

Stratmann, J. W. (2003). "Long distance run in the wound response - jasmonic acid is pulling ahead." Trends in Plant Science **8**(6): 247-250.

Strawn, M. A., S. K. Marr, K. Inoue, N. Inada, C. Zubieta and M. C. Wildermuth (2007). "Arabidopsis isochorismate synthase functional in pathogen-induced salicylate biosynthesis exhibits properties consistent with a role in diverse stress responses." J Biol Chem **282**(8): 5919-5933.

Subramanian, S., U. H. Cho, C. Keyes and O. Yu (2009). "Distinct changes in soybean xylem sap proteome in response to pathogenic and symbiotic microbe interactions." Bmc Plant Biology **9**.

Takeuchi, O. and S. Akira (2010). "Pattern recognition receptors and inflammation." Cell **140**(6): 805-820.

Tena, G., M. Boudsocq and J. Sheen (2011). "Protein kinase signaling networks in plant innate immunity." Curr Opin Plant Biol **14**(5): 519-529.

Thaler, J. S., P. T. Humphrey and N. K. Whiteman (2012). "Evolution of jasmonate and salicylate signal crosstalk." Trends in Plant Science **17**(5): 260-270.

Thibaud, M. C., S. Gineste, L. Nussaume and C. Robaglia (2004). "Sucrose increases pathogenesis-related PR-2 gene expression in Arabidopsis thaliana through an SA-dependent but NPR1-independent signaling pathway." Plant Physiology and Biochemistry **42**(1): 81-88.

Thomma, B. P. H. J., T. Nurnberger and M. H. A. J. Joosten (2011). "Of PAMPs and Effectors: The Blurred PTI-ETI Dichotomy." Plant Cell **23**(1): 4-15.

Thomma, B. P. H. J., I. A. M. A. Penninckx, W. F. Broekaert and B. P. A. Cammue (2001). "The complexity of disease signaling in Arabidopsis." Current Opinion in Immunology **13**(1): 63-68.

Torres, M. A., J. D. Jones and J. L. Dangl (2006). "Reactive oxygen species signaling in response to pathogens." Plant Physiol **141**(2): 373-378.

Truman, W., M. H. Bennett, I. Kubigsteltig, C. Turnbull and M. Grant (2007). "Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates." Proc Natl Acad Sci U S A **104**(3): 1075-1080.

Tsuda, K. and F. Katagiri (2010). "Comparing signaling mechanisms engaged in pattern-triggered and effector-triggered immunity." Current Opinion in Plant Biology **13**(4): 459-465.

Tsuda, K., M. Sato, T. Stoddard, J. Glazebrook and F. Katagiri (2009). "Network properties of robust immunity in plants." PLoS Genet **5**(12): e1000772.

Uhlig, S., M. Jestoi, A. Kristin Knutsen and B. T. Heier (2006). "Multiple regression analysis as a tool for the identification of relations between semi-quantitative LC-MS data and cytotoxicity of extracts of the fungus Fusarium avenaceum (syn. F. arthrosporioides)." Toxicon **48**(5): 567-579.

Van Damme, E. J., A. Barre, P. Rouge and W. J. Peumans (2004). "Cytoplasmic/nuclear plant lectins: a new story." Trends Plant Sci **9**(10): 484-489.

Van Damme, E. J., N. Lannoo and W. J. Peumans (2008). "Plant Lectins." Advances in Botanical Research **48**(Elsevier Ltd, San Diego): 107-209.

Van der Biezen, E. A. and J. D. Jones (1998). "Plant disease-resistance proteins and the gene-for-gene concept." Trends Biochem Sci **23**(12): 454-456.

Vandenborre, G., G. Smagghe and E. J. M. Van Damme (2011). "Plant lectins as defense proteins against phytophagous insects." Phytochemistry **72**(13): 1538-1550.

Vasconcelos, I. M. and J. T. A. Oliveira (2004). "Antinutritional properties of plant lectins." Toxicon **44**(4): 385-403.

Vernooij, B., L. Friedrich, A. Morse, R. Reist, R. Kolditz-Jawhar, E. Ward, S. Uknes, H. Kessmann and J. Ryals (1994). "Salicylic Acid Is Not the Translocated Signal Responsible for Inducing Systemic Acquired Resistance but Is Required in Signal Transduction." Plant Cell **6**(7): 959-965.

Vidal, S., I. P. deLeon, J. Denecke and E. T. Palva (1997). "Salicylic acid and the plant pathogen Erwinia carotovora induce defense genes via antagonistic pathways." Plant Journal **11**(1): 115-123.

Vlot, A. C., D. A. Dempsey and D. F. Klessig (2009). "Salicylic Acid, a multifaceted hormone to combat disease." Annu Rev Phytopathol **47**: 177-206.

Vlot, A. C., D. F. Klessig and S. W. Park (2008). "Systemic acquired resistance: the elusive signal(s)." Current Opinion in Plant Biology **11**(4): 436-442.

Wang, D., N. D. Weaver, M. Kesarwani and X. Dong (2005). "Induction of protein secretory pathway is required for systemic acquired resistance." Science **308**(5724): 1036-1040.

Wang, S., W. E. Durrant, J. Q. Song, N. W. Spivey and X. N. Dong (2010). "Arabidopsis BRCA2 and RAD51 proteins are specifically involved in defense gene transcription during plant immune responses." Proceedings of the National Academy of Sciences of the United States of America **107**(52): 22716-22721.

Wang, Y. H., C. Gehring and H. R. Irving (2011). "Plant natriuretic peptides are apoplastic and paracrine stress response molecules." Plant Cell Physiol **52**(5): 837-850.

Washburn, M. P., D. Wolters and J. R. Yates (2001). "Large-scale analysis of the yeast proteome by multidimensional protein identification technology." Nature Biotechnology **19**(3): 242-247.

Wasternack, C., I. Stenzel, B. Hause, G. Hause, C. Kutter, H. Maucher, J. Neumerkel, I. Feussner and O. Miersch (2006). "The wound response in tomato - Role of jasmonic acid." Journal of Plant Physiology **163**(3): 297-306.

Wesley, S. V., C. A. Helliwell, N. A. Smith, M. B. Wang, D. T. Rouse, Q. Liu, P. S. Gooding, S. P. Singh, D. Abbott, P. A. Stoutjesdijk, S. P. Robinson, A. P. Gleave, A. G. Green and P. M. Waterhouse (2001). "Construct design for efficient, effective and high-throughput gene silencing in plants." Plant J **27**(6): 581-590.

Wiermer, M., B. J. Feys and J. E. Parker (2005). "Plant immunity: the EDS1 regulatory node." Curr Opin Plant Biol **8**(4): 383-389.

Wildermuth, M. C., J. Dewdney, G. Wu and F. M. Ausubel (2001). "Isochorismate synthase is required to synthesize salicylic acid for plant defence." Nature **414**(6863): 562-565.

Xia, Y., H. Suzuki, J. Borevitz, J. Blount, Z. Guo, K. Patel, R. A. Dixon and C. Lamb (2004). "An extracellular aspartic protease functions in Arabidopsis disease resistance signaling." EMBO J **23**(4): 980-988.

Xiao, W., J. Sheen and J. C. Jang (2000). "The role of hexokinase in plant sugar signal transduction and growth and development." Plant Mol Biol **44**(4): 451-461.

Yalpani, N., J. Leon, M. A. Lawton and I. Raskin (1993). "Pathway of Salicylic Acid Biosynthesis in Healthy and Virus-Inoculated Tobacco." Plant Physiol **103**(2): 315-321.

Yamaguchi, Y. and A. Huffaker (2011). "Endogenous peptide elicitors in higher plants." Current Opinion in Plant Biology **14**(4): 351-357.

Yao, X., W. Xiong, T. T. Ye and Y. Wu (2012). "Overexpression of the aspartic protease ASPG1 gene confers drought avoidance in Arabidopsis." Journal of Experimental Botany **63**(7): 2579-2593.

Yin, Y., J. Chory and D. Baulcombe (2005). "RNAi in transgenic plants." Curr Protoc Mol Biol **Chapter 26**: Unit 26 26.

Yuan, S. and H. H. Lin (2008). "Role of salicylic acid in plant abiotic stress." Z Naturforsch C **63**(5-6): 313-320.

Zhang, B., K. Ramonell, S. Somerville and G. Stacey (2002). "Characterization of early, chitin-induced gene expression in Arabidopsis." Mol Plant Microbe Interact **15**(9): 963-970.

Zhang, J., F. Shao, Y. Li, H. Cui, L. Chen, H. Li, Y. Zou, C. Long, L. Lan, J. Chai, S. Chen, X. Tang and J. M. Zhou (2007). "A Pseudomonas syringae effector inactivates MAPKs to suppress PAMP-induced immunity in plants." Cell Host Microbe **1**(3): 175-185.

Zhang, W., X. Yang, D. Qiu, L. Guo, H. Zeng, J. Mao and Q. Gao (2010). "PeaT1-induced systemic acquired resistance in tobacco follows salicylic acid-dependent pathway." Mol Biol Rep **38**(4): 2549-2556.

Zhu-Salzman, K., R. E. Shade, H. Koiwa, R. A. Salzman, M. Narasimhan, I. A. Bressan, P. M. Hasegawa and L. L. Murdock (1998). "Carbohydrate binding and resistance to proteolysis control insecticidal activity of Griffonia simplicifolia lectin II." Proceedings of the National Academy of Sciences of the United States of America **95**(25): 15123-15128.

Zipfel, C. (2008). "Pattern-recognition receptors in plant innate immunity." Curr Opin Immunol **20**(1): 10-16.

Zuo, J. R., Q. W. Niu and N. H. Chua (2000). "An estrogen receptor-based transactivator XVE mediates highly inducible gene expression in transgenic plants." Plant Journal **24**(2): 265-273.

Zwicker, S., S. Mast, V. Stos, A. J. Pfitzner and U. M. Pfitzner (2007). "Tobacco NIMIN2 proteins control PR gene induction through transient repression early in systemic acquired resistance." Mol Plant Pathol **8**(4): 385-400.

CHAPTER 6: SUPPLEMENT

Primer used in this thesis.

Table 25: Primers used for T-DNA insertion KO lines.

Acronym	Name	Sequence (5´- 3´)	fragment size	Annealing temperature
HB-P1	LBb1 (pBIN-pROK2)	GCGTGGACCGCTTGCTGCAACT		64°C /60°C
HB-P2	SALK_030762 LP	TCCGTGAAGAAAACAAACAAAG		
HB-P3	SALK_030762 RP	GAGACGAAACCCCATTCTCTC	1081bp	64°C
HB-P4	SALK_036814 LP	TTGGGATGCAAAGCAAATTAC		
HB-P5	SALK_036814 RP	CTTTCTCAGCAACAACGGAAG	1190bp	60°C

The suitable annealing temperature of the LBb1 (pBIN-pROK2) primer depended on the RP primer used for the selection process as specified in the method section.

Table 26: Primers used for the generation of over-expression constructs in recombinant expression vectors using the Gateway™ technology (CACC cloning sequence required for the entry of the fragments into pENTR™/D-TOPO® in italics).

Acronym	Name	Sequence (5´- 3´)	fragment size	Annealing temperature
HB-P6	*At*AED1 neighbor F	*CACC*ATGAGCATAAATAGGAATCTG		
HB-P7	*At*AED1 neighbor R	ACTACACCCGTTTGGAGCAAAC	1425bp	55.3°C
HB-P8	*At*AED4 F	*CACC*GTGAGATCGATGGAGAGTTAC		
HB-P9	*At*AED4 R	AAGCTGTGCTAATTGCGAGATATC	1095bp	60°C
HB-P10	*At*AED5 F	*CACC*ATGGAAACTCTTTTCCACAC		
HB-P11	*At*AED5 R	AAGATTCAAAGATGACGCCGTC	1140bp	57°C
HB-P12	*At*AED9-1 F	*CACC*TCCACACTTGAATCACA		
HB-P13	*At*AED9-1 R	GTTTTCAAACGACCAGCTC	816bp	58°C
HB-P14	*At*AED9-2 F	*CACC*TCAATCTTTCAATCAC		
HB-P15	*At*AED9-2 R	GTTGTTTTTGGCGGCGTTT	831bp	52°C

HB-P16	*At*AED9-3 F	*CACC*ATGAAGATTCATAAACTCTGTTTTCTT		
HB-P17	*At*AED9-3 R	GATTCTCTTGGCACTGTTCTGGA	825bp	52.3°C

Table 27: Primers used for the generation of the RNAi silencing constructs using the Gateway™ technology (CACC cloning sequence required for the entry of the fragments into pENTR™/D-TOPO® in italics).

Acronym	Name	Sequence (5´- 3´)	fragment size	Annealing temperature
HB-P18	RNAi_2er_ *At*AED9-1 F	*CACC*CTCGAGGGATCCACTTCGATTCCTTC GATGGC		
HB-P19	RNAi_2er_ *At*AED9-1 R	GATATCCGCCATCTGAAGCACCGGGCTGG GACAATGACGAAG	279bp	59.5°C
HB-P20	RNAi_2er_ *At*AED9-2 F	CTTCGTCATTGTCCCAGCCCGGTGCTTCAG ATGGCGGATATC		
HB-P21	RNAi_2er_ *At*AED9-2 R	GGTACCAAGCTTTCAGGCGCGAGTGTAACC	319bp	59.5°C
HB-P22	RNAi_3er_ *At*AED9-1 F	*CACC*CTCGAGGGATCCACTTCGATTCCTTC GATGGC		
HB-P23	RNAi_3er_ *At*AED9-1 R	CCGCCATCTGAAGCACCGGGGCTGGGACA ATGACGAAG	279bp	59.5°C
HB-P24	RNAi_3er_ *At*AED9-2 F	CTTCGTCATTGTCCCAGCCCCGGTGCTTCA GATGGCGG		
HB-P25	RNAi_3er_ *At*AED9-2 R	CGGCGGAAGTGGTGTGAGCTTCAGGCGCG AGTGTAACCG	319bp	59.5°C
HB-P26	RNAi_3er_ *At*AED9-3 F	CGGTTACACTCGCGCCTGAAGCTCACACCA CTTCCGCCG		
HB-P27	RNAi_3er_ *At*AED9-3 R	GGTACCAAGCTTAAGCAAGGCCGTGACCA GGG	284bp	59.5°C

Table 28: Primers used for qPCR-analysis.

Acronym	Name	Sequence (5´- 3´)	fragment size	Target-gene
HB-P28	qP *At*AED1 F	ATTATGCTTTGCGTATGTCTCAATTGG		
HB-P29	qP *At*AED1 R	GGACTCCACACGTGCTTGATCG	261bp	*AtAED1*
HB-P30	qP *At*AED1n F	CGATGGAGCTCAAGAAAGAGAGACTGA		
HB-P31	qP *At*AED1n R	GCGAGCCTGGTCGAGTCTTAGGA	214bp	*AtAED1 neighbor*

HB-P32	qP *At*AED4 F	TCGACGTTCTCACTGAGCTACTTGGA		
HB-P33	qP *At*AED4 R	CCACCACTTGTGCCACTGTGTTC	201bp	*AtAED4*
HB-P34	qP *At*AED5 F	GCGGTTTTCAACTTCGGAGACTCTAA		
HB-P35	qP *At*AED5 R	GGGCTATAGGATGCAGCATTTGC	281bp	*AtAED5*
HB-P36	qP *At*AED6 F	GTGGCTGGAACGACCCGGATA		
HB-P37	qP *At*AED6 R	TGGTCCTGCCCAAAC CTCGA	251bp	*AtAED6*
HB-P38	qP *At*AED7 F	CCGCTTACGATGTCGATAATTGG		
HB-P39	qP *At*AED7 R	CAAGACATCTACTGAATCACAATCTGAAA	263bp	*AtAED7*
HB-P40	qP *At*AED8 F	ATGCTGCAAAGAAAACTGTTGT		
HB-P41	qP *At*AED8 R	TACCATGTCTCTGGTAGTCT	190bp	*AtAED8*
HB-P42	qP *At*AED9-1 F	TTTGGAGCTGGTCGTTTGAA		
HB-P43	qP *At*AED9-1 R (3´UTR)	TTTGGAGCTGGTCGTTTGAA	99bp	*AtAED9-1*
HB-P44	qP *At*AED9-2 F	TTTGGAGCTGGTCGTTTGAA		
HB-P45	qP *At*AED9-2 R (3´UTR)	AGTTACCACTGAGTAGTATG	127bp	*AtAED9-2*
HB-P46	qP *At*AED9-3 F	TGAGTAAACAGCAGTTACGA		
HB-P47	qP *At*AED9-3 R (3´UTR)	TGACGCCATCAGAAGCAGGA	166bp	*AtAED9-3*
HB-P48	qP *At*AED10 F	GCCTGAGGAAGCAACGATAG		
HB-P49	qP *At*AED10 R	TCCAAGCACGACTACACAGC	184bp	*AtAED10*
HB-P50	qP *At*AED11 F	TGGTGTCAGATTCCGGTACA		
HB-P51	qP *At*AED11 R	CATCCCTGAACCTTCCTTGA	191bp	*PR2*
HB-P52	qP *At*AED12 F	GGAGAGGAACGTGCCATAAA		
HB-P53	qP *At*AED12 R	TAACCGAACTCGAGGCTGAT	227bp	*AtAED12*
HB-P54	qP *At*AED13 F	ATCGGGAGATTGCAAATACG		
HB-P55	qP *At*AED13 R	GCGTAGCTATAGGCGTCAGG	228bp	*PR5*
HB-P56	qP *At*AED14 F	AGCTGCTCAAGGAAAAGCTG		
HB-P57	qP *At*AED14 R	AATGCATCGAACCCTGTACC	151bp	*AtAED14*
HB-P58	qP *At*AED15 F	GGAACGGTGACACCTGACTT		

HB-P59	qP *At*AED15 R	CTTGGCTGGTCCATCAATTT	219bp	*AtAED15*
HB-P60	qP *At*AED16 F	CCGTGTGGGTTCTGATTTCT		
HB-P61	qP *At*AED16 R	GTCACAGTCTTGGAGCACGA	163bp	*AtAED16*
HB-P62	qP *At*AED17 F	CAAGGAACCAACCTTCTCCA		
HB-P63	qP *At*AED17 R	GCCTCCTCTTCTGCAACAAC	182bp	*AtAED17*
HB-P64	qP *At*AED18 F	TAGCAAGATTGGCTGACGTG		
HB-P65	qP *At*AED18 R	AGCAAATAGCAGCACCGAGT	240bp	*AtAED18*
HB-P66	qP PR1 F	CTACGCAGAACAACTAAGAGGCAAC		*Arabidopsis*
HB-P67	qP PR1 R	TTGGCACATCCGAGTCTCACTG	220bp	*PR 1*
HB-P68	qP Ubi F	AGATCCAGGACAAGGTATTC		*Arabidopsis*
HB-P69	qP Ubi R	CGCAGGACCAAGTGAAGAGTAG	150bp	*Ubiquitin*
HB-P70	qP Tub9 F	GTACCTTGAAGCTTGCTAATCCTA		*Arabidopsis*
HB-P71	qP Tub9 R	GTCAAAGGTGCAAAACCAAC	186bp	*Tubulin*
HB-P72	PR1tab F qP	ACCTCGTACATTCTCATG		*N. tabacum*
HB-P73	PR1tab R qP	ACCTGTCCTTGTGCACAA	145bp	*PR1*
HB-P74	EF1 Tabak F qP	TTCTCGACTGCCACACTTCCA		*N. tabacum*
HB-P75	EF1 Tabak R qP	TAACTGTCCGCAAGACCATTCCT	163bp	*EF1α*

All primers used for qPCR had the same annealing temperature of 60°C. The reverse primers used for *AtAED9-1*, *AtAED9-2* and *AtAED9-3* were designed in the 3´untranslated region (3´UTR) in order to get specific amplification of all three target genes due to their similarity in their coding sequence.

Table 29: Overview of the AED proteins identified using the 2D-gel approach.

Protein	Acronym	Locus number	Predicted size [kDa]
Eukaryotic aspartyl protease family protein	*At*AED1	*At5G10760*	*49.4*
ASPG 1 (aspartic pro-tease in guard cell 1)	*At*AED2	*At3G18490*	*53.2*
Eukaryotic aspartyl protease family protein	*At*AED3	*At1G09750*	*47.6*

Table 19 continued	***Acronym***	**Locus Number**	**Predicted size [kDa]**
GDSL motif lipase	*At*AED4	*At1G29660*	*40.1*
GDSL motif lipase	*At*AED5	*At3G05180*	*42.3*
α-galactosidase	*At*AED6	*At5G08380*	*45.7*
β-xylosidase	*At*AED7	*At5G64570*	*84.3*

Table 30: Complete list of ICPL-detected proteins in three biological repetitions.

Proteins in bold were selected for a part of the integrated protein list. Heavy/light values in bold show the significantly regulated proteins. Proteins highlighted in red are significantly down regulated in the *eds1-2* mutant in comparison to *Arabidopsis* wild type.

Accession	MW [kDa]	Description	Heavy/Light	Heavy/Light Counts	Heavy/Light Variability [%]
gi116830447	17,7	unknown [Arabidopsis thaliana]	**8,867**	1;-;-	
gi15225974	17,7	PR1 (PATHOGENESIS-RELATED GENE 1) [Arabidopsis thaliana]	**5,897**	1;1;-	33,4
gi166916096	**17,9**	**beta-1,3-glucanase 2 [Arabidopsis thaliana]**	**5,615**	**3;1;1**	**41,7**
gi4375833	**77,0**	**receptor serine/threonine kinase-like protein [Arabidopsis thaliana]**	**2,920**	**1;1;1**	**30,0**
gi15222089	**25,2**	**PR5 (PATHOGENESIS-RELATED GENE 5) [Arabidopsis thaliana]**	**2,903**	**4;4;6**	**46,0**
gi18398655	82,9	SBT3.5; identical protein binding / serine-type endopeptidase [Arabidopsis thaliana]	**2,712**	1;-;-	
gi4185132	**14,0**	**hypothetical protein [Arabidopsis thaliana]**	**2,654**	**2;2;1**	**19,6**
gi15224308	**29,8**	**chitinase, putative [Arabidopsis thaliana]**	**2,335**	**2;3;1**	**47,3**
gi62321746	**41,2**	**pectin methylesterase like protein [Arabidopsis thaliana]**	**2,310**	**9;12;5**	**37,3**
gi145334541	41,2	SCPL34; serine-type carboxypeptidase [Arabidopsis thaliana]	**2,308**	-;-;1	
gi15235401	**24,1**	**ATGSTF2 (GLUTATHIONE S-TRANSFERASE PHI 2); glutathione binding / glutathione transferase [Arabidopsis thaliana]**	**2,268**	**3;2;2**	**65,9**
gi30692067	**38,1**	**NIT1; indole-3-acetonitrile nitrilase/ indole-3-acetonitrile nitrile hydratase/ nitrilase [Arabidopsis thaliana]**	**2,243**	**17;10;5**	**18,8**
gi51968436	19,1	putative protein [Arabidopsis thaliana]	**2,196**	-;1;-	
gi25373339	24,5	unknown protein F14G6.5 [imported] - Arabidopsis thaliana	**2,085**	-;6;4	34,5
gi15242724	**30,1**	**legume lectin family protein [Arabidopsis thaliana]**	**2,004**	**4;5;4**	**30,0**
gi15236416	**29,7**	**EP1; protein kinase [Arabidopsis thaliana]**	**1,960**	**2;2;1**	**28,3**
gi42571953	43,5	BGLU46 (BETA GLUCOSIDASE 46); catalytic/ cation binding / hydrolase, hydrolyzing O-glycosyl compounds [Arabidopsis thaliana]	1,854	-;-;3	
gi15231255	63,3	chaperonin, putative [Arabidopsis thaliana]	1,815	-;3;2	104,0
gi15233499	44,2	leucine-rich repeat family protein / extensin family protein [Arabidopsis thaliana]	1,807	-;1;-	
gi42562700	41,8	jacalin lectin family protein [Arabidopsis thaliana]	1,751	1;-;-	
gi42568045	45,9	pectinacetylesterase family protein [Arabidopsis thaliana]	1,741	-;1;1	21,6
gi15232216	29,7	tRNA-binding region domain-containing protein [Arabidopsis thaliana]	1,723	-;3;-	

gi15218039	25,5	immunophilin / FKBP-type peptidyl-prolyl cis-trans isomerase family protein [Arabidopsis thaliana]	1,693	-;4;-	
gi6056400	42,7	Similar to anther-specific proline-rich protein APG [Arabidopsis thaliana]	1,689	-;1;-	
gi16930701	61,1	AT3g14240/MLN21_2 [Arabidopsis thaliana]	1,648	1;-;-	
gi15237225	44,1	HCF136; protein binding [Arabidopsis thaliana]	1,635	-;1;3	31,7
gi79325087	48,4	MEE58 (MATERNAL EFFECT EMBRYO ARREST 58); adenosylhomocysteinase/ copper ion binding [Arabidopsis thaliana]	1,633	3;5;1	40,3
gi30684671	25,5	ankyrin repeat family protein [Arabidopsis thaliana]	1,627	-;1;-	
gi15241812	34,1	peroxidase, putative [Arabidopsis thaliana]	1,603	6;5;7	27,1
gi8778617	116,6	F5M15.5 [Arabidopsis thaliana]	1,553	15;-;10	16,2
gi222424707	53,0	AT1G20620 [Arabidopsis thaliana]	1,553	15;-;10	16,2
gi110738836	33,8	putative beta-1,3-glucanase [Arabidopsis thaliana]	1,537	-;2;-	
gi15218574	46,7	eukaryotic translation initiation factor 4A, putative / eIF-4A, putative [Arabidopsis thaliana]	1,535	-;2;-	
gi18391179	15,4	DNA-binding protein-related [Arabidopsis thaliana]	1,530	-;3;-	
gi15233111	39,9	CYSC1 (CYSTEINE SYNTHASE C1); L-3-cyanoalanine synthase/ cysteine synthase [Arabidopsis thaliana]	1,523	-;-;1	
gi145334581	26,6	peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	1,522	-;1;-	
gi15223164	20,6	ARFA1D; GTP binding / phospholipase activator/ protein binding [Arabidopsis thaliana]	1,517	-;5;4	70,0
gi79325123	23,6	photosystem II reaction center PsbP family protein [Arabidopsis thaliana]	1,504	3;6;4	19,4
gi15228102	30,7	29 kDa ribonucleoprotein, chloroplast, putative / RNA-binding protein cp29, putative [Arabidopsis thaliana]	1,501	-;13;12	44,6
gi15228202	35,0	esterase/lipase/thioesterase family protein [Arabidopsis thaliana]	1,487	1;-;-	
gi15233841	28,6	ATEXLA2 (ARABIDOPSIS THALIANA EXPANSIN-LIKE A2) [Arabidopsis thaliana]	1,479	-;2;-	
gi38566630	18,5	At1g30730 [Arabidopsis thaliana]	1,472	-;1;-	
gi15236625	48,6	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein [Arabidopsis thaliana]	1,469	-;1;-	
gi7525040	53,9	ATP synthase CF1 beta subunit [Arabidopsis thaliana]	1,468	1;1;-	64,7
gi159163414	15,5	Chain A, Solution Structure Of Hypothetical Protein F20o9.120 From Arabidopsis Thaliana	1,464	-;5;6	32,1
gi15235745	57,4	SHM1 (SERINE TRANSHYDROXYMETHYLTRANSFERASE 1); glycine hydroxymethyltransferase/ poly(U) binding [Arabidopsis thaliana]	1,456	6;17;4	27,4
gi15224088	35,1	translation initiation factor 3 (IF-3) family protein [Arabidopsis thaliana]	1,451	-;2;3	44,0
gi15241478	61,4	FAD-binding domain-containing protein [Arabidopsis thaliana]	1,451	4;6;2	14,9
gi6715645	75,2	T25K16.8 [Arabidopsis thaliana]	1,448	5;1;-	39,6
gi15220512	40,1	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	1,445	3;7;2	10,5
gi15229095	38,8	PRXCB (PEROXIDASE CB); peroxidase [Arabidopsis thaliana]	1,444	1;-;-	
gi15221123	25,3	SEC22; transporter [Arabidopsis thaliana]	1,444	-;1;-	
gi15235421	39,9	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	1,443	1;-;1	27,3
gi15242951	22,4	ATPase activator/ chaperone binding [Arabidopsis thaliana]	1,441	-;3;3	35,6

gi4559346	18,8	similar to early nodulins [Arabidopsis thaliana]	1,429	4;5;2	21,7
gi18403247	27,8	receptor protein kinase-related [Arabidopsis thaliana]	1,428	2;3;2	20,9
gi332646778	28,1	Peptidyl-prolyl cis-trans isomerase CYP20-3 [Arabidopsis thaliana]	1,427	41;47;49	14,4
gi30696347	25,6	thylakoid lumenal 17.4 kDa protein, chloroplast [Arabidopsis thaliana]	1,427	6;9;8	7,6
gi18379115	18,8	photosystem II family protein [Arabidopsis thaliana]	1,419	5;9;5	29,3
gi15237615	34,9	peroxidase, putative [Arabidopsis thaliana]	1,410	3;2;1	6,8
gi30693595	27,7	RNA recognition motif (RRM)-containing protein [Arabidopsis thaliana]	1,410	-;2;2	33,1
gi681904	34,5	RNA-binding protein cp29 [Arabidopsis thaliana]	1,407	-;6;3	47,1
gi10441352	51,9	ARF GAP-like zinc finger-containing protein ZIGA3 [Arabidopsis thaliana]	1,402	2;2;1	21,6
gi4583542	23,0	16 kDa polypeptide of oxygen-evolving complex [Arabidopsis thaliana]	1,394	6;8;8	9,5
gi469193	56,1	amidophosphoribosyltransferase [Arabidopsis thaliana]	1,393	-;1;-	
gi15229656	53,2	aspartyl protease family protein [Arabidopsis thaliana]	1,390	8;7;11	7,0
gi186478207	23,7	PSBP-1 (PHOTOSYSTEM II SUBUNIT P-1); poly(U) binding [Arabidopsis thaliana]	1,390	14;22;21	17,6
gi18415779	22,0	MSRB2 (methionine sulfoxide reductase B 2); peptide-methionine-(S)-S-oxide reductase [Arabidopsis thaliana]	1,387	-;1;1	12,0
gi9758142	60,5	pectinesterase like protein [Arabidopsis thaliana]	1,381	2;1;1	16,4
gi15236514	54,7	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein [Arabidopsis thaliana]	1,376	-;2;2	12,2
gi15241571	53,6	scpl35 (serine carboxypeptidase-like 35); serine-type carboxypeptidase [Arabidopsis thaliana]	1,376	6;7;6	14,1
gi15234681	32,1	XTH18 (XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 18); hydrolase, acting on glycosyl bonds / hydrolase, hydrolyzing O-glycosyl compounds / xyloglucan:xyloglucosyl transferase [Arabidopsis thaliana]	1,374	-;2;1	17,3
gi227204285	30,8	AT4G37530 [Arabidopsis thaliana]	1,372	1;-;-	
gi15227259	18,5	ROC3; peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	1,366	9;6;9	22,2
gi1246399	56,9	catalase [Arabidopsis thaliana]	1,364	2;-;4	48,6
gi42572967	60,2	FAD-binding domain-containing protein [Arabidopsis thaliana]	1,361	22;22;22	10,6
gi4204267	35,3	Unknown protein [Arabidopsis thaliana]	1,360	1;3;7	8,2
gi18252211	46,1	aspartate--tRNA ligase - like protein [Arabidopsis thaliana]	1,359	2;-;-	
gi145358477	26,8	unknown protein [Arabidopsis thaliana]	1,351	2;1;2	43,0
gi18379072	46,1	extracellular dermal glycoprotein, putative / EDGP, putative [Arabidopsis thaliana]	1,342	3;10;3	14,4
gi15236544	18,9	plastocyanin-like domain-containing protein [Arabidopsis thaliana]	1,341	2;1;-	15,3
gi15222954	33,7	CDSP32 (CHLOROPLASTIC DROUGHT-INDUCED STRESS PROTEIN OF 32 KD) [Arabidopsis thaliana]	1,340	1;3;3	56,4
gi20198164	17,8	hypothetical protein [Arabidopsis thaliana]	1,338	4;3;6	20,2
gi15224305	23,5	immunophilin / FKBP-type peptidyl-prolyl cis-trans isomerase family protein [Arabidopsis thaliana]	1,335	2;7;11	18,7
gi62318887	17,6	glyceraldehyde 3-phosphate dehydrogenase A subunit [Arabidopsis thaliana]	1,329	2;4;2	11,2
gi15220770	36,2	ACO2 (ACC OXIDASE 2); 1-aminocyclopropane-1-carboxylate oxidase	1,324	1;-;-	

		[Arabidopsis thaliana]			
gi21537233	45,8	EDGP precursor [Arabidopsis thaliana]	1,324	19;26;23	9,6
gi30697435	83,7	SVL2 (SHV3-LIKE 2); glycerophosphodiester phosphodiesterase/ kinase [Arabidopsis thaliana]	1,321	6;8;6	13,5
gi8671874	29,1	Contains a weak similarity to chalcone--flavonone isomerase from Pueraria lobata gi|Q43056 and containes fanconi anaemia group C protein PF|02106 domain [Arabidopsis thaliana]	1,320	2;5;5	34,7
gi332193488	52,6	Leucine-rich repeat (LRR) family protein [Arabidopsis thaliana]	1,317	6;11;9	33,8
gi15229384	43,9	CSP41A (CHLOROPLAST STEM-LOOP BINDING PROTEIN OF 41 KDA); mRNA binding / poly(U) binding [Arabidopsis thaliana]	1,314	4;6;3	38,2
gi15231354	93,6	BGAL1 (Beta galactosidase 1); beta-galactosidase/ catalytic/ cation binding / heme binding / peroxidase/ sugar binding [Arabidopsis thaliana]	1,312	13;16;8	24,7
gi15218836	20,4	disease resistance-responsive family protein [Arabidopsis thaliana]	1,306	-;1;1	4,5
gi3850621	42,1	putative RNA binding protein [Arabidopsis thaliana]	1,306	1;8;3	42,5
gi15238971	25,3	ATVAMP713 (VESICLE-ASSOCIATED MEMBRANE PROTEIN 713) [Arabidopsis thaliana]	1,301	-;1;1	18,1
gi15228229	30,5	legume lectin family protein [Arabidopsis thaliana]	1,300	5;12;12	10,8
gi15239000	29,0	33 kDa secretory protein-related [Arabidopsis thaliana]	1,297	2;2;2	15,7
gi185177593	60,7	Chain A, Crystal Structure Of A Cell-Wall Invertase (D239a) From Arabidopsis Thaliana In Complex With Sucrose	1,292	4;6;10	9,7
gi15224916	23,1	RAB6A; GTP binding / protein binding [Arabidopsis thaliana]	1,291	-;-;1	
gi15224349	54,1	unknown protein [Arabidopsis thaliana]	1,290	1;-;-	
gi18416643	36,2	aldose 1-epimerase family protein [Arabidopsis thaliana]	1,289	15;13;14	2,6
gi12321045	102,2	xylan endohydrolase isoenzyme, putative [Arabidopsis thaliana]	1,285	1;2;-	36,7
gi15242933	22,5	YKT61 [Arabidopsis thaliana]	1,285	-;2;3	41,5
gi42571483	29,7	oxidoreductase NAD-binding domain-containing protein [Arabidopsis thaliana]	1,282	-;1;1	21,3
gi15237763	19,8	FTRA1 (ferredoxin/thioredoxin reductase subunit A (variable subunit) 1); catalytic/ ferredoxin reductase/ ferredoxin:thioredoxin reductase/ lipoate synthase [Arabidopsis thaliana]	1,280	-;2;3	38,1
gi30688093	18,8	glutaredoxin family protein [Arabidopsis thaliana]	1,276	2;4;3	21,5
gi6041844	17,3	unknown protein [Arabidopsis thaliana]	1,275	-;1;-	
gi14594802	41,8	translation initiation factor eIF-4A1 [Arabidopsis thaliana]	1,275	1;2;-	28,9
gi15237739	21,9	ROC7; peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	1,271	3;-;-	
gi15230324	35,0	PSBO2 (PHOTOSYSTEM II SUBUNIT O-2); oxygen evolving/ poly(U) binding [Arabidopsis thaliana]	1,270	1;2;4	27,3
gi30695023	47,1	unknown protein [Arabidopsis thaliana]	1,268	-;-;1	
gi18423214	103,4	CLPC1; ATP binding / ATP-dependent peptidase/ ATPase [Arabidopsis thaliana]	1,267	2;2;3	13,7
gi15224321	30,4	chitinase, putative [Arabidopsis thaliana]	1,263	-;2;1	32,5
gi13877871	52,8	putative serine carboxypeptidase II [Arabidopsis thaliana]	1,254	4;3;7	7,0
gi14030667	34,2	At1g03480/F21B7_24 [Arabidopsis thaliana]	1,254	4;4;1	17,1

gi15221446	88,5	subtilase family protein [Arabidopsis thaliana]	1,250	7;5;5	11,4
gi21594055	40,1	lipase/hydrolase, putative [Arabidopsis thaliana]	1,249	1;-;-	
gi15218202	83,8	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]	1,249	15;23;15	4,8
gi15236687	25,0	ATVAMP711 (ARABIDOPSIS THALIANA VESICLE-ASSOCIATED MEMBRANE PROTEIN 711) [Arabidopsis thaliana]	1,246	2;-;-	
gi15226462	45,9	pectinacetylesterase, putative [Arabidopsis thaliana]	1,244	1;-;-	
gi145329985	20,6	CYP5 (CYCLOPHILIN 5); peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	1,243	4;5;-	3,3
gi92090800	25,0	RecName: Full=Thylakoid lumenal 19 kDa protein, chloroplastic; AltName: Full=P19; Flags: Precursor	1,240	4;10;7	9,2
gi332004470	27,9	Peptidyl-prolyl cis-trans isomerase CYP20-2 [Arabidopsis thaliana]	1,238	7;12;10	15,9
gi9558599	54,2	Nearly identical to vacuolar ATP synthase subunit B (V-atpase B subunit)(V-atpase 57 KD subunit) from Arabidopsis thaliana gi|137465 and is a member of ATP synthase alpha/beta PF|00006 family and contains an ATP synthase beta chain PF|01038 domain. ESTs gb|F14109, gb|AA650677, gb|N65767, gb|BE038735, gb|T88157, gb|F14079, gb|H76885, gb|N96777, gb|T14042 come from this gene	1,238	-;3;3	7,5
gi7715602	47,0	F20B17.14 [Arabidopsis thaliana]	1,237	8;9;10	9,5
gi15233245	25,6	PPL1 (PsbP-like protein 1); calcium ion binding [Arabidopsis thaliana]	1,236	7;11;11	8,1
gi42569717	83,2	ceramidase family protein [Arabidopsis thaliana]	1,235	3;1;5	14,6
gi18402763	39,8	phospholipase C/ phosphoric diester hydrolase [Arabidopsis thaliana]	1,234	2;1;-	0,9
gi15234637	24,6	PSBQ-2; calcium ion binding [Arabidopsis thaliana]	1,230	4;8;8	9,2
gi30680681	72,3	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]	1,229	4;4;3	12,4
gi42572317	34,7	unknown protein [Arabidopsis thaliana]	1,229	19;23;16	22,9
gi15221119	44,4	aminomethyltransferase, putative [Arabidopsis thaliana]	1,228	2;18;24	16,1
gi15222218	40,4	MAP1C (METHIONINE AMINOPEPTIDASE 1B); aminopeptidase/ metalloexopeptidase [Arabidopsis thaliana]	1,226	5;10;5	41,2
gi15242674	11,7	glutaredoxin, putative [Arabidopsis thaliana]	1,224	-;1;-	
gi18397757	25,1	secretory protein, putative [Arabidopsis thaliana]	1,223	2;-;1	29,0
gi30689979	93,7	FUC95A; 1,2-alpha-L-fucosidase [Arabidopsis thaliana]	1,222	16;10;9	5,1
gi79319775	52,3	BGLU18 (BETA GLUCOSIDASE 18); catalytic/ cation binding / hydrolase, hydrolyzing O-glycosyl compounds [Arabidopsis thaliana]	1,216	2;-;-	
gi21450872	39,8	unknown protein [Arabidopsis thaliana]	1,216	12;10;13	39,4
gi18406661	23,8	thylakoid lumenal 15 kDa protein, chloroplast [Arabidopsis thaliana]	1,215	5;8;8	4,7
gi110742393	19,8	thioredoxin m4 [Arabidopsis thaliana]	1,214	-;6;6	24,6
gi15228051	40,7	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	1,213	-;-;2	
gi30699237	28,5	thylakoid lumenal 29.8 kDa protein [Arabidopsis thaliana]	1,212	2;3;2	14,6
gi55670202	13,6	Chain A, Crystal Structure Of Atfkbp13	1,211	1;2;4	5,7
gi7413642	30,5	putative protein [Arabidopsis thaliana]	1,210	2;2;3	18,6
gi15810383	36,6	unknown protein [Arabidopsis thaliana]	1,210	3;3;4	42,5
gi15240641	31,8	31 kDa ribonucleoprotein, chloroplast, putative / RNA-binding protein RNP-T,	1,209	4;1;4	23,2

		putative / RNA-binding protein 1/2/3, putative / RNA-binding protein cp31, putative [Arabidopsis thaliana]			
gi15229105	65,3	pectinesterase family protein [Arabidopsis thaliana]	1,209	10;10;5	15,1
gi25347778	51,6	hypothetical protein At2g17760 [imported] - Arabidopsis thaliana	1,207	1;-;-	
gi15238891	32,4	XTH20 (XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 20); hydrolase, acting on glycosyl bonds / hydrolase, hydrolyzing O-glycosyl compounds / xyloglucan:xyloglucosyl transferase [Arabidopsis thaliana]	1,207	-;-;1	
gi7262685	62,7	Weak similarity to glyoxal oxidase (glx2) from Phanerochaete chrysosporium gb|L47287 [Arabidopsis thaliana]	1,201	3;3;4	15,7
gi18396920	50,2	unknown protein [Arabidopsis thaliana]	1,199	-;1;-	
gi15232477	24,3	AtRABA1g (Arabidopsis Rab GTPase homolog A1g); GTP binding [Arabidopsis thaliana]	1,198	1;-;-	
gi15234942	28,6	peptide methionine sulfoxide reductase, putative [Arabidopsis thaliana]	1,197	8;10;10	19,9
gi79577675	19,6	unknown protein [Arabidopsis thaliana]	1,197	3;2;1	14,2
gi18396193	80,0	subtilase family protein [Arabidopsis thaliana]	1,196	6;12;17	23,1
gi18412657	30,4	RRF (RIBOSOME RECYCLING FACTOR, CHLOROPLAST PRECURSOR) [Arabidopsis thaliana]	1,196	2;2;5	34,5
gi4584540	64,9	putative protein [Arabidopsis thaliana]	1,195	1;1;-	27,5
gi15222942	68,2	calcineurin-like phosphoesterase family protein [Arabidopsis thaliana]	1,193	6;2;-	4,1
gi110741949	63,4	ubiquitin carboxyl-terminal hydrolase like protein [Arabidopsis thaliana]	1,192	1;-;-	
gi15228618	51,4	aspartyl protease family protein [Arabidopsis thaliana]	1,191	2;1;3	10,0
gi23397307	48,2	unknown protein [Arabidopsis thaliana]	1,191	1;1;-	1,2
gi18395285	15,1	DNA-binding protein-related [Arabidopsis thaliana]	1,191	1;-;-	
gi79327847	41,9	3-isopropylmalate dehydrogenase, chloroplast, putative [Arabidopsis thaliana]	1,190	-;-;1	
gi2244964	31,1	hypothetical protein [Arabidopsis thaliana]	1,188	-;1;1	54,2
gi240256136	58,5	enzyme inhibitor/ pectinesterase [Arabidopsis thaliana]	1,185	1;-;-	
gi15238328	52,8	scpl42 (serine carboxypeptidase-like 42); serine-type carboxypeptidase [Arabidopsis thaliana]	1,185	5;9;8	12,2
gi6630454	200,7	F23N19.18 [Arabidopsis thaliana]	1,184	-;1;1	16,4
gi2827712	31,2	endoxyloglucan tranferase-like protein [Arabidopsis thaliana]	1,183	11;13;9	19,1
gi15226361	101,9	leucine-rich repeat protein kinase, putative [Arabidopsis thaliana]	1,182	2;4;2	14,1
gi9802764	60,4	Putative pectinesterase [Arabidopsis thaliana]	1,181	24;33;23	2,1
gi15225026	44,2	AGT (ALANINE:GLYOXYLATE AMINOTRANSFERASE); alanine-glyoxylate transaminase/ serine-glyoxylate transaminase/ serine-pyruvate transaminase [Arabidopsis thaliana]	1,181	-;1;1	18,0
gi30695409	40,9	ACAT2 (ACETOACETYL-COA THIOLASE 2); acetyl-CoA C-acetyltransferase/ catalytic [Arabidopsis thaliana]	1,178	1;1;-	56,1
gi30690925	57,6	pectinesterase family protein [Arabidopsis thaliana]	1,177	4;4;5	15,8
gi18403243	32,6	PDLP3 (PLASMODESMATA-LOCATED PROTEIN 3) [Arabidopsis thaliana]	1,171	3;-;-	
gi18411711	79,9	transketolase, putative [Arabidopsis thaliana]	1,168	20;18;11	19,7
gi2760345	34,1	ubiquitin [Arabidopsis thaliana]	1,167	-;8;8	11,7

gi18398912	51,7	disease resistance protein-related / LRR protein-related [Arabidopsis thaliana]	1,167	30;44;34	15,2
gi15226983	33,8	33 kDa ribonucleoprotein, chloroplast, putative / RNA-binding protein cp33, putative [Arabidopsis thaliana]	1,166	3;4;8	40,7
gi62321216	38,0	ketol-acid reductoisomerase [Arabidopsis thaliana]	1,162	2;-;-	
gi1255987	29,9	GF14chi isoform [Arabidopsis thaliana]	1,159	2;-;-	
gi15232828	24,5	immunophilin, putative / FKBP-type peptidyl-prolyl cis-trans isomerase, putative [Arabidopsis thaliana]	1,158	4;5;6	22,8
gi18405061	31,1	thylakoid lumen 18.3 kDa protein [Arabidopsis thaliana]	1,154	-;1;2	25,7
gi13926291	35,1	AT5g66570/K1F13_25 [Arabidopsis thaliana]	1,154	1;2;3	35,5
gi18401075	74,8	unknown protein [Arabidopsis thaliana]	1,151	-;1;2	29,2
gi18415308	42,1	pectinacetylesterase, putative [Arabidopsis thaliana]	1,150	11;19;12	4,7
gi15242465	33,4	AtPPa6 (Arabidopsis thaliana pyrophosphorylase 6); inorganic diphosphatase/ pyrophosphatase	1,149	-;1;-	
gi2062167	34,8	Proline-rich protein APG isolog [Arabidopsis thaliana]	1,149	-;3;2	27,5
gi15222981	60,0	sks5 (SKU5 Similar 5); copper ion binding / oxidoreductase [Arabidopsis thaliana]	1,148	37;48;43	19,6
gi145329204	32,3	TIM (TRIOSEPHOSPHATE ISOMERASE); catalytic/ triose-phosphate isomerase [Arabidopsis thaliana]	1,148	-;2;-	
gi11692884	11,7	AT4g28750 [Arabidopsis thaliana]	1,148	3;5;4	2,0
gi22331102	29,7	legume lectin family protein [Arabidopsis thaliana]	1,147	5;10;12	20,0
gi15237736	84,3	XYL4; hydrolase, hydrolyzing O-glycosyl compounds / xylan 1,4-beta-xylosidase [Arabidopsis thaliana]	1,146	27;32;32	11,2
gi336390	42,8	glyceraldehyde 3-phosphate dehydrogenase B subunit [Arabidopsis thaliana]	1,142	-;-;1	
gi18415500	91,8	subtilase family protein [Arabidopsis thaliana]	1,139	5;6;4	11,1
gi15229018	50,1	GSA2 (glutamate-1-semialdehyde 2,1-aminomutase 2); catalytic/ glutamate-1-semialdehyde 2,1-aminomutase/ pyridoxal phosphate binding / transaminase [Arabidopsis thaliana]	1,139	4;5;3	15,7
gi30692256	10,2	GR-RBP8; RNA binding / nucleic acid binding / nucleotide binding [Arabidopsis thaliana]	1,139	5;8;10	11,5
gi145334317	72,2	amidase family protein [Arabidopsis thaliana]	1,139	1;-;-	
gi15228814	18,9	ROC2 (ROTAMASE CYCLOPHILIN 2); cyclosporin A binding / peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	1,138	1;2;2	8,6
gi6630558	67,7	putative pectinesterase [Arabidopsis thaliana]	1,138	4;3;2	26,6
gi240254668	55,0	unknown protein [Arabidopsis thaliana]	1,136	1;1;1	13,0
gi15226573	65,5	NIR1 (NITRITE REDUCTASE 1); ferredoxin-nitrate reductase/ nitrite reductase (NO-forming) [Arabidopsis thaliana]	1,136	18;13;5	28,4
gi20196872	20,7	expressed protein [Arabidopsis thaliana]	1,135	2;-;-	
gi15232559	60,8	GR (GLUTATHIONE REDUCTASE); ATP binding / glutathione-disulfide reductase [Arabidopsis thaliana]	1,135	1;2;1	38,8
gi227206222	10,8	AT2G21660 [Arabidopsis thaliana]	1,134	2;3;2	19,1
gi4406810	41,2	unknown protein [Arabidopsis thaliana]	1,129	-;1;1	41,6
gi30699276	44,1	peptidoglycan-binding LysM domain-containing protein [Arabidopsis thaliana]	1,128	5;4;3	9,5
gi145330372	16,4	ubiquitin-conjugating enzyme family protein [Arabidopsis thaliana]	1,127	-;4;2	13,7
gi222424554	37,8	AT4G22670 [Arabidopsis thaliana]	1,127	1;-;-	

gi110736691	42,0	isoleucyl-tRNA synthetase [Arabidopsis thaliana]	1,124	2;-;-	
gi42573816	43,5	5-azacytidine resistance protein -related [Arabidopsis thaliana]	1,124	-;1;-	
gi27752799	47,5	ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Arabidopsis thaliana]	1,121	159;170;1	15,0
gi30697303	15,4	ADF4 (ACTIN DEPOLYMERIZING FACTOR 4); actin binding [Arabidopsis thaliana]	1,120	1;2;2	10,3
gi186510990	82,0	BGAL2 (beta-galactosidase 2); beta-galactosidase/ catalytic/ cation binding [Arabidopsis thaliana]	1,118	9;9;1	21,9
gi15220514	39,8	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	1,118	16;18;16	14,1
gi15219257	25,7	PAB1 (PROTEASOME SUBUNIT PAB1); endopeptidase/ peptidase/ threonine-type endopeptidase [Arabidopsis thaliana]	1,117	2;-;1	23,2
gi30697215	60,0	HEXO3 (BETA-HEXOSAMINIDASE 3); beta-N-acetylhexosaminidase/ hexosaminidase [Arabidopsis thaliana]	1,112	1;3;6	5,7
gi14194127	60,4	AT4g20860/T13K14_20 [Arabidopsis thaliana]	1,111	20;19;14	5,2
gi222424967	61,1	AT4G39640 [Arabidopsis thaliana]	1,111	4;3;4	16,4
gi16323089	35,8	At3g12148/T23B7.11 [Arabidopsis thaliana]	1,108	1;-;-	
gi5051777	25,7	putative protein [Arabidopsis thaliana]	1,108	1;1;1	10,9
gi15230764	65,8	ACLB-1; ATP citrate synthase [Arabidopsis thaliana]	1,108	5;9;4	29,5
gi332657799	12,0	early nodulin-like protein 19 [Arabidopsis thaliana]	1,107	2;3;1	5,8
gi15028379	48,4	putative beta-1,3-glucanase [Arabidopsis thaliana]	1,105	2;-;1	1,1
gi15220874	22,1	trypsin and protease inhibitor family protein / Kunitz family protein [Arabidopsis thaliana]	1,105	2;5;6	15,8
gi6562297	57,8	putative protein [Arabidopsis thaliana]	1,105	-;1;-	
gi15233416	60,1	FAD-binding domain-containing protein [Arabidopsis thaliana]	1,104	24;30;22	11,6
gi15232503	45,4	aspartyl protease family protein [Arabidopsis thaliana]	1,100	21;27;27	18,4
gi4163997	101,6	alpha-xylosidase precursor [Arabidopsis thaliana]	1,099	4;10;9	14,2
gi15241573	41,0	HEMC (HYDROXYMETHYLBILANE SYNTHASE); hydroxymethylbilane synthase [Arabidopsis thaliana]	1,098	20;19;24	32,7
gi18403435	43,3	unknown protein [Arabidopsis thaliana]	1,098	3;6;3	14,2
gi18414469	176,6	GLU1 (GLUTAMATE SYNTHASE 1); glutamate synthase (ferredoxin) [Arabidopsis thaliana]	1,098	22;43;23	26,5
gi330252447	50,6	leucine aminopeptidase 1 [Arabidopsis thaliana]	1,097	-;-;1	
gi576937	19,5	Meri-5 [Arabidopsis thaliana]	1,097	4;2;5	22,6
gi24417282	51,8	unknown [Arabidopsis thaliana]	1,097	11;9;-	7,8
gi15237059	51,6	ATRABE1B (ARABIDOPSIS RAB GTPASE HOMOLOG E1B); GTP binding / GTPase/ translation elongation factor [Arabidopsis thaliana]	1,094	4;4;4	34,6
gi15239867	83,5	BXL1 (BETA-XYLOSIDASE 1); hydrolase, hydrolyzing O-glycosyl compounds [Arabidopsis thaliana]	1,094	22;28;18	29,4
gi15224111	53,0	ALDH11A3; 3-chloroallyl aldehyde dehydrogenase/ glyceraldehyde-3-phosphate dehydrogenase (NADP+) [Arabidopsis thaliana]	1,090	-;1;2	37,7
gi22326920	67,9	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]	1,087	4;4;10	7,7
gi110742034	113,7	putative glycine dehydrogenase [Arabidopsis thaliana]	1,085	4;3;3	16,4
gi83754491	53,3	Chain A, Crystallographic Structure Of Arabidopsis Thaliana Threonine Synthase	1,082	1;-;-	

		Complexed With Pyridoxal Phosphate And S-Adenosylmethionine			
gi42568662	18,9	RNA and export factor-binding protein, putative [Arabidopsis thaliana]	1,081	1;-;1	12,7
gi30696930	52,1	monodehydroascorbate reductase, putative [Arabidopsis thaliana]	1,080	6;11;7	19,4
gi14596025	112,9	P-Protein - like protein [Arabidopsis thaliana]	1,080	13;10;7	16,7
gi15237128	35,6	peroxidase, putative [Arabidopsis thaliana]	1,079	1;-;-	
gi30682530	27,7	alanine racemase family protein [Arabidopsis thaliana]	1,078	-;2;2	26,6
gi18404496	34,9	binding / catalytic/ coenzyme binding [Arabidopsis thaliana]	1,078	9;9;15	30,9
gi227202728	28,7	AT1G20020 [Arabidopsis thaliana]	1,077	4;4;7	32,1
gi757534	78,2	subtilisin-like protease [Arabidopsis thaliana]	1,077	32;30;27	12,2
gi145326672	29,4	lactoylglutathione lyase, putative / glyoxalase I, putative [Arabidopsis thaliana]	1,076	1;2;3	38,7
gi62321345	23,3	glutamate--ammonia ligase [Arabidopsis thaliana]	1,074	1;1;-	10,4
gi15239944	96,2	catalytic [Arabidopsis thaliana]	1,073	6;4;3	5,5
gi18395044	43,5	LYM1 (LYSM DOMAIN GPI-ANCHORED PROTEIN 1 PRECURSOR) [Arabidopsis thaliana]	1,071	1;-;-	
gi18420570	34,6	haloacid dehalogenase-like hydrolase family protein [Arabidopsis thaliana]	1,071	1;2;4	46,6
gi2275219	105,6	unknown protein [Arabidopsis thaliana]	1,071	4;4;3	18,7
gi15235997	32,0	XTR6 (XYLOGLUCAN ENDOTRANSGLYCOSYLASE 6); hydrolase, acting on glycosyl bonds / hydrolase, hydrolyzing O-glycosyl compounds / xyloglucan:xyloglucosyl transferase [Arabidopsis thaliana]	1,069	2;-;-	
gi18394414	31,3	uridylyltransferase-related [Arabidopsis thaliana]	1,068	-;2;2	12,7
gi8778976	51,7	Strong similarity to alanine aminotransferase from Zea mays gb|AF055898. It contains an aminotransferases class-I domain PF|00155. ESTs gb|AV546814, gb|AV519234, gb|AV536176, gb|AV537339, gb|AV544878, gb|AV532954, gb|AV553416, gb|AV519356, gb|AV537898, gb|AI999107, gb|AV545731, gb|AI995660, gb|AV550634, gb|AV536556, gb|AV531066, gb|T45832, gb|AV549979, gb|T04047, gb|AV549129, gb|T88429 and gb|AI993829 come from this gene. This gene is cut off [Arabidopsis thaliana]	1,068	5;2;2	17,4
gi18397406	46,3	pectinacetylesterase, putative [Arabidopsis thaliana]	1,067	6;10;5	10,4
gi3273755	16,7	copper/zinc superoxide dismutase [Arabidopsis thaliana]	1,067	1;-;2	19,0
gi18395017	77,2	unknown protein [Arabidopsis thaliana]	1,065	55;83;69	12,1
gi15232671	91,8	PLDALPHA1 (PHOSPHOLIPASE D ALPHA 1); phospholipase D [Arabidopsis thaliana]	1,065	11;5;4	14,4
gi1944432	47,6	ribulosebisphosphate carboxylase [Arabidopsis thaliana]	1,061	153;167;-	18,0
gi18401659	22,2	CSD2 (COPPER/ZINC SUPEROXIDE DISMUTASE 2); superoxide dismutase [Arabidopsis thaliana]	1,061	-;-;1	
gi110740925	33,1	tryptophan synthase alpha chain [Arabidopsis thaliana]	1,060	-;-;1	
gi15226185	42,3	fructose-bisphosphate aldolase, putative [Arabidopsis thaliana]	1,060	-;3;1	27,5
gi15242357	42,0	pectinacetylesterase, putative [Arabidopsis thaliana]	1,059	5;6;6	7,8
gi110736982	24,5	hypothetical protein [Arabidopsis thaliana]	1,057	1;1;2	22,8
gi18405982	19,5	avirulence-responsive protein, putative / avirulence induced gene (AIG) protein,	1,053	-;-;3	

		putative [Arabidopsis thaliana]			
gi15242781	32,1	TCH4 (Touch 4); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase [Arabidopsis thaliana]	1,053	5;3;-	21,1
gi2961390	95,2	beta-galactosidase like protein [Arabidopsis thaliana]	1,051	2;-;-	
gi15232626	59,7	PYK10; beta-glucosidase/ copper ion binding / fucosidase/ hydrolase, hydrolyzing O-glycosyl compounds [Arabidopsis thaliana]	1,051	3;4;2	35,7
gi5123926	25,4	putative protein [Arabidopsis thaliana]	1,051	-;2;3	21,6
gi18422451	90,6	subtilase family protein [Arabidopsis thaliana]	1,049	2;-;-	
gi15242897	83,0	BGAL10 (beta-galactosidase 10); beta-galactosidase/ catalytic/ cation binding [Arabidopsis thaliana]	1,049	8;6;3	16,3
gi22326796	115,8	glycosyl hydrolase family 38 protein [Arabidopsis thaliana]	1,047	23;36;27	5,1
gi15242458	39,6	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	1,047	1;-;-	
gi9759559	46,9	nucleoid DNA-binding-like protein [Arabidopsis thaliana]	1,046	1;3;5	9,5
gi51969896	35,7	hypothetical protein [Arabidopsis thaliana]	1,046	1;10;6	30,1
gi62319313	10,3	eukaryotic translation initiation factor 3 subunit like protein [Arabidopsis thaliana]	1,046	2;-;4	5,2
gi14190449	13,1	At1g14320/F14L17_28 [Arabidopsis thaliana]	1,042	1;-;-	
gi15233538	14,0	PFN2 (PROFILIN 2); actin binding / protein binding [Arabidopsis thaliana]	1,041	-;3;2	29,0
gi15241897	63,7	SYNC1; ATP binding / aminoacyl-tRNA ligase/ asparagine-tRNA ligase/ aspartate-tRNA ligase/ nucleic acid binding / nucleotide binding [Arabidopsis thaliana]	1,041	2;-;-	
gi15242430	24,7	mob1/phocein family protein [Arabidopsis thaliana]	1,038	3;-;2	6,2
gi30688284	72,3	glycosyl hydrolase family protein 27 / alpha-galactosidase family protein / melibiase family protein [Arabidopsis thaliana]	1,036	8;8;3	22,5
gi22326803	57,5	SET domain-containing protein [Arabidopsis thaliana]	1,036	-;1;-	
gi15240520	84,1	SVL1 (SHV3-LIKE 1); glycerophosphodiester phosphodiesterase/ phosphoric diester hydrolase [Arabidopsis thaliana]	1,032	5;6;4	17,5
gi15222729	63,8	CPN60B (CHAPERONIN 60 BETA); ATP binding / protein binding [Arabidopsis thaliana]	1,031	4;5;9	58,9
gi18417320	103,7	emb2726 (embryo defective 2726); RNA binding / translation elongation factor [Arabidopsis thaliana]	1,031	10;5;3	51,5
gi79313229	38,1	(S)-2-hydroxy-acid oxidase, peroxisomal, putative / glycolate oxidase, putative / short chain alpha-hydroxy acid oxidase, putative [Arabidopsis thaliana]	1,030	-;3;1	6,9
gi30687995	49,1	RCA (RUBISCO ACTIVASE); ADP binding / ATP binding / enzyme regulator/ ribulose-1,5-bisphosphate carboxylase/oxygenase activator [Arabidopsis thaliana]	1,029	2;2;1	41,6
gi15234551	65,6	SKU5; copper ion binding / oxidoreductase [Arabidopsis thaliana]	1,027	15;16;10	8,2
gi15242495	60,4	pectinesterase family protein [Arabidopsis thaliana]	1,027	4;2;3	19,0
gi15237540	47,1	UVR8 (UVB-RESISTANCE 8); chromatin binding / guanyl-nucleotide exchange factor [Arabidopsis thaliana]	1,026	1;-;2	3,1
gi15226718	27,2	ATEXPA8 (ARABIDOPSIS THALIANA EXPANSIN A8) [Arabidopsis thaliana]	1,025	2;3;-	19,4
gi18404212	54,3	ALDH2C4; 3-chloroallyl aldehyde dehydrogenase/ aldehyde dehydrogenase (NAD)/ coniferyl-aldehyde dehydrogenase [Arabidopsis thaliana]	1,022	2;1;1	12,8
gi222423050	39,9	AT3G51800 [Arabidopsis thaliana]	1,022	2;3;-	10,3

gi62321401	28,2	enolase [Arabidopsis thaliana]	1,021	5;6;5	10,7
gi222424560	34,3	AT4G19420 [Arabidopsis thaliana]	1,020	1;-;1	40,9
gi79318801	33,0	lipase, putative [Arabidopsis thaliana]	1,015	6;6;4	14,7
gi21555870	46,7	Eukaryotic initiation factor 4A, putative [Arabidopsis thaliana]	1,014	1;-;-	
gi18402886	29,0	haloacid dehalogenase-like hydrolase family protein [Arabidopsis thaliana]	1,013	1;4;1	46,3
gi15238933	56,6	ADG1 (ADP GLUCOSE PYROPHOSPHORYLASE 1); glucose-1-phosphate adenylyltransferase [Arabidopsis thaliana]	1,010	8;4;3	17,5
gi18399513	33,8	PSB29 [Arabidopsis thaliana]	1,007	1;7;5	30,1
gi145327229	17,6	TIR (TOLL/INTERLEUKIN-1 RECEPTOR-LIKE); transmembrane receptor [Arabidopsis thaliana]	1,006	6;8;4	9,1
gi15219234	68,8	VHA-A (VACUOLAR ATP SYNTHASE SUBUNIT A); ATP binding / hydrogen ion transporting ATP synthase, rotational mechanism / hydrolase, acting on acid anhydrides, catalyzing transmembrane movement of substances / proton-transporting ATPase, rotational mechanism [Arabidopsis thaliana]	1,005	1;1;1	26,6
gi15239154	101,1	alpha-glucosidase 1 (AGLU1) [Arabidopsis thaliana]	1,005	16;15;15	4,3
gi18402122	16,8	ATPH1 (ARABIDOPSIS THALIANA PLECKSTRIN HOMOLOGUE 1); phosphoinositide binding [Arabidopsis thaliana]	1,004	1;3;3	14,9
gi15450421	40,4	At1g02500/T14P4_22 [Arabidopsis thaliana]	1,003	-;-;2	
gi42573724	36,4	malate dehydrogenase (NADP), chloroplast, putative [Arabidopsis thaliana]	1,003	-;-;1	
gi30692666	44,7	AtGUS3 (Arabidopsis thaliana glucuronidase 3); beta-glucuronidase	0,999	11;9;9	4,1
gi42561840	107,6	glycoside hydrolase family 2 protein [Arabidopsis thaliana]	0,995	3;8;2	4,2
gi4510395	91,8	putative beta-galactosidase [Arabidopsis thaliana]	0,993	26;26;21	8,6
gi15234354	43,2	SAM-2 (S-ADENOSYLMETHIONINE SYNTHETASE 2); copper ion binding / methionine adenosyltransferase [Arabidopsis thaliana]	0,993	-;-;2	
gi186479101	76,6	amine oxidase/ copper ion binding / quinone binding [Arabidopsis thaliana]	0,993	13;8;11	32,8
gi15237656	32,7	EXL2 (EXORDIUM LIKE 2) [Arabidopsis thaliana]	0,993	6;5;5	46,5
gi21592865	34,4	unknown [Arabidopsis thaliana]	0,993	6;11;5	5,2
gi15235714	33,7	xyloglucan:xyloglucosyl transferase, putative / xyloglucan endotransglycosylase, putative / endo-xyloglucan transferase, putative [Arabidopsis thaliana]	0,991	23;25;21	5,0
gi6041854	17,8	unknown protein [Arabidopsis thaliana]	0,990	1;-;-	
gi26185954	55,7	cytosolic phosphoglucomutase [Arabidopsis thaliana]	0,988	1;-;-	
gi18395849	26,8	FLA7 (FASCICLIN-LIKE ARABINOOGALACTAN 7) [Arabidopsis thaliana]	0,987	5;3;3	7,1
gi30687121	81,5	BGAL12 (beta-galactosidase 12); beta-galactosidase/ catalytic/ cation binding [Arabidopsis thaliana]	0,986	1;3;2	14,0
gi110739416	79,7	beta-galactosidase like protein [Arabidopsis thaliana]	0,986	24;35;26	8,4
gi15225605	34,3	EXGT-A1 (ENDOXYLOGLUCAN TRANSFERASE); hydrolase, acting on glycosyl bonds / xyloglucan:xyloglucosyl transferase [Arabidopsis thaliana]	0,985	37;43;23	18,8
gi15240474	25,1	RAN3 (RAN GTPASE 3); GTP binding / GTPase/ protein binding [Arabidopsis thaliana]	0,985	22;22;19	10,7
gi21593731	53,2	putative serine carboxypeptidase II [Arabidopsis thaliana]	0,982	3;6;6	3,4

gi15223944	15,1	CSD1 (COPPER/ZINC SUPEROXIDE DISMUTASE 1); superoxide dismutase [Arabidopsis thaliana]	0,982	-;-;1	
gi15241844	73,6	BIP1; ATP binding [Arabidopsis thaliana]	0,981	3;-;1	1,2
gi6642633	48,5	putative beta-1,3-glucanase precursor [Arabidopsis thaliana]	0,980	1;-;-	
gi109157627	111,0	Chain A, Crystal Structure Of Presequence Protease Prep From Arabidopsis Thaliana	0,978	3;-;1	33,4
gi30686575	20,2	unknown protein [Arabidopsis thaliana]	0,978	2;-;-	
gi18410417	63,3	glucose-methanol-choline (GMC) oxidoreductase family protein [Arabidopsis thaliana]	0,974	1;-;-	
gi11228579	36,6	aspartate-semialdehyde dehydrogenase precursor [Arabidopsis thaliana]	0,972	1;2;2	14,8
gi15239061	30,5	PAF1; endopeptidase/ peptidase/ threonine-type endopeptidase [Arabidopsis thaliana]	0,972	-;2;-	
gi30687308	17,1	antioxidant/ oxidoreductase [Arabidopsis thaliana]	0,971	-;1;1	4,4
gi15241168	49,6	TUA3; structural constituent of cytoskeleton [Arabidopsis thaliana]	0,970	1;-;-	
gi15236768	38,3	fructose-bisphosphate aldolase, putative [Arabidopsis thaliana]	0,970	-;3;3	9,1
gi15220176	48,8	WIN1 (HOPW1-1-INTERACTING 1); N2-acetyl-L-ornithine:2-oxoglutarate 5-aminotransferase/ catalytic/ pyridoxal phosphate binding / transaminase [Arabidopsis thaliana]	0,969	-;3;2	23,5
gi30683851	85,0	NAI2 [Arabidopsis thaliana]	0,968	6;2;3	19,5
gi15232929	35,3	peroxidase, putative [Arabidopsis thaliana]	0,968	7;9;5	16,9
gi15234781	18,4	ROC1 (ROTAMASE CYP 1); peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	0,966	5;9;10	15,5
gi22331145	66,0	transducin family protein / WD-40 repeat family protein [Arabidopsis thaliana]	0,966	4;6;1	16,8
gi6041822	49,2	unknown protein [Arabidopsis thaliana]	0,966	3;3;2	9,7
gi15227954	27,8	EMB2296 (embryo defective 2296); structural constituent of ribosome [Arabidopsis thaliana]	0,965	1;-;1	5,7
gi15220315	24,9	ATVAMP726 [Arabidopsis thaliana]	0,964	-;-;2	
gi30683758	57,2	ATFUC1 (alpha-L-fucosidase 1); alpha-L-fucosidase [Arabidopsis thaliana]	0,964	3;3;2	8,7
gi145360782	38,2	binding / catalytic [Arabidopsis thaliana]	0,961	-;1;1	21,9
gi15231611	115,1	glycosyl hydrolase family 38 protein [Arabidopsis thaliana]	0,961	1;-;-	
gi15233349	98,1	aconitate hydratase, cytoplasmic / citrate hydro-lyase / aconitase (ACO) [Arabidopsis thaliana]	0,960	7;4;3	16,7
gi186522071	39,1	ATP binding / ATP-dependent helicase/ helicase/ nucleic acid binding [Arabidopsis thaliana]	0,960	1;1;-	13,4
gi15237614	36,1	peroxidase, putative [Arabidopsis thaliana]	0,959	1;2;-	29,8
gi21592566	38,6	putative malonyl-CoA:Acyl carrier protein transacylase [Arabidopsis thaliana]	0,958	2;3;4	13,7
gi145329995	18,6	ATGSTF9 (GLUTATHIONE S-TRANSFERASE PHI 9); glutathione peroxidase/ glutathione transferase [Arabidopsis thaliana]	0,956	3;5;3	44,5
gi15231674	29,6	GUN4; enzyme binding / tetrapyrrole binding [Arabidopsis thaliana]	0,956	-;-;4	
gi79318406	48,5	GGT1 (GLUTAMATE:GLYOXYLATE AMINOTRANSFERASE); L-alanine:2-oxoglutarate aminotransferase/ glycine:2-oxoglutarate aminotransferase [Arabidopsis thaliana]	0,955	12;17;15	4,7
gi18405794	107,8	VLN2 (VILLIN 2); actin binding [Arabidopsis thaliana]	0,955	2;-;2	32,5
gi15235889	25,1	PBA1; endopeptidase/ peptidase/ threonine-type endopeptidase [Arabidopsis thaliana]	0,954	-;1;-	

gi3096942	22,7	putative protein [Arabidopsis thaliana]	0,953	-;-;2	
gi15226834	61,3	FAD-binding domain-containing protein [Arabidopsis thaliana]	0,952	3;2;-	0,2
gi30690089	51,4	TGG1 (THIOGLUCOSIDE GLUCOHYDROLASE 1); hydrolase, hydrolyzing O-glycosyl compounds / thioglucosidase [Arabidopsis thaliana]	0,949	25;39;26	20,7
gi15237747	19,6	C/VIF2 (CELL WALL / VACUOLAR INHIBITOR OF FRUCTOSIDASE 2); enzyme inhibitor/ pectinesterase/ pectinesterase inhibitor [Arabidopsis thaliana]	0,948	1;-;-	
gi30698086	65,0	ASN2 (ASPARAGINE SYNTHETASE 2); asparagine synthase (glutamine-hydrolyzing) [Arabidopsis thaliana]	0,947	3;-;-	
gi15450453	17,7	AT5g66550/K1F13_22 [Arabidopsis thaliana]	0,947	2;-;-	
gi15232373	39,8	leucine-rich repeat family protein [Arabidopsis thaliana]	0,945	6;14;7	13,9
gi79329027	54,1	CAC2; acetyl-CoA carboxylase/ biotin carboxylase [Arabidopsis thaliana]	0,943	2;5;-	3,2
gi15239146	64,4	ATNADP-ME2 (NADP-malic enzyme 2); malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+)/ malic enzyme/ oxidoreductase, acting on NADH or NADPH, NAD or NADP as acceptor [Arabidopsis thaliana]	0,943	4;2;1	37,7
gi2584721	71,8	sulfite reductase [Arabidopsis thaliana]	0,942	10;9;6	11,7
gi18413740	26,5	Clp amino terminal domain-containing protein [Arabidopsis thaliana]	0,942	-;3;4	0,1
gi110740777	39,2	glutamine synthetase like protein [Arabidopsis thaliana]	0,941	-;1;1	2,6
gi18408627	34,2	haloacid dehalogenase-like hydrolase family protein [Arabidopsis thaliana]	0,941	4;4;8	23,7
gi15242420	29,5	EXL4 (EXORDIUM LIKE 4) [Arabidopsis thaliana]	0,940	2;1;3	26,9
gi15237088	20,9	disease resistance-responsive family protein / dirigent family protein [Arabidopsis thaliana]	0,939	1;2;1	40,5
gi15237350	39,8	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	0,938	-;4;-	
gi332645337	18,3	high mobility group protein B1 [Arabidopsis thaliana]	0,938	1;-;-	
gi15218869	45,7	isocitrate dehydrogenase, putative / NADP+ isocitrate dehydrogenase, putative [Arabidopsis thaliana]	0,936	6;14;9	19,9
gi10178279	40,2	alpha-galactosidase-like protein [Arabidopsis thaliana]	0,936	4;2;4	13,2
gi475720	18,3	RNA-binding protein 3 [Arabidopsis thaliana]	0,936	17;5;7	14,7
gi15239684	57,6	APL1 (ADP GLUCOSE PYROPHOSPHORYLASE LARGE SUBUNIT 1); glucose-1-phosphate adenylyltransferase [Arabidopsis thaliana]	0,936	3;7;6	14,0
gi15222848	36,9	GAPC2 (GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE C2); NAD or NADH binding / binding / catalytic/ glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)/ glyceraldehyde-3-phosphate dehydrogenase [Arabidopsis thaliana]	0,935	1;1;1	23,1
gi18395019	78,4	unknown protein [Arabidopsis thaliana]	0,933	1;-;-	
gi13926229	14,7	F1O19.10/F1O19.10 [Arabidopsis thaliana]	0,930	2;8;2	11,5
gi18404397	47,0	DJ-1 family protein [Arabidopsis thaliana]	0,930	-;-;2	
gi25408221	141,9	probable myosin heavy chain [imported] - Arabidopsis thaliana	0,929	2;-;2	58,4
gi15220463	34,9	RCI3 (RARE COLD INDUCIBLE GENE 3); peroxidase [Arabidopsis thaliana]	0,929	1;-;-	
gi1498198	28,9	2-Cys peroxiredoxin bas1 [Arabidopsis thaliana]	0,928	7;5;7	13,5
gi30698124	27,8	GRF8 (GENERAL REGULATORY FACTOR 8); protein binding / protein phosphorylated amino acid binding [Arabidopsis thaliana]	0,928	1;1;-	64,2

gi10242314	42,8	12-oxo-phytodienoate reductase [Arabidopsis thaliana]	0,927	-;-;1	
gi13377778	43,4	fasciclin-like arabinogalactan-protein 2 [Arabidopsis thaliana]	0,927	6;7;5	1,4
gi15227981	38,4	fructose-bisphosphate aldolase, putative [Arabidopsis thaliana]	0,927	-;1;-	
gi30682028	35,8	Aha1 domain-containing protein [Arabidopsis thaliana]	0,926	3;3;4	40,8
gi17473683	19,1	glycolate oxidase [Arabidopsis thaliana]	0,926	-;2;-	
gi15226314	62,0	CPN60A (CHAPERONIN-60ALPHA); ATP binding / protein binding [Arabidopsis thaliana]	0,926	8;8;17	48,2
gi3913518	37,5	RecName: Full=SAL1 phosphatase; AltName: Full=3'(2'),5'-bisphosphate nucleotidase 1; AltName: Full=3'(2'),5'-bisphosphonucleoside 3'(2')-phosphohydrolase 1; AltName: Full=DPNPase 1; AltName: Full=Inositol-1,4-bisphosphate 1-phosphatase 1; AltName: Full=Inositol polyphosphate 1-phosphatase 1; Short=IPPase 1; AltName: Full=Protein FIERY 1	0,924	3;1;1	3,9
gi12230525	151,7	RecName: Full=Probable phosphoribosylformylglycinamidine synthase, chloroplastic; Short=FGAM synthase; Short=FGAMS; AltName: Full=Formylglycinamide ribotide amidotransferase; Short=FGARAT; AltName: Full=Formylglycinamide ribotide synthetase; Flags: Precursor	0,924	3;-;1	32,9
gi18401116	51,6	NSP1 (NITRILE SPECIFIER PROTEIN 1) [Arabidopsis thaliana]	0,924	13;3;4	27,5
gi30696056	93,8	LOS1; copper ion binding / translation elongation factor/ translation factor, nucleic acid binding [Arabidopsis thaliana]	0,923	37;44;30	31,0
gi15220216	36,2	ANNAT1 (ANNEXIN ARABIDOPSIS 1); ATP binding / calcium ion binding / calcium-dependent phospholipid binding / copper ion binding / peroxidase/ protein homodimerization [Arabidopsis thaliana]	0,922	8;2;2	37,0
gi62320725	47,8	cysteine proteinase RD21A [Arabidopsis thaliana]	0,918	-;3;2	5,1
gi227204143	43,9	AT1G57720 [Arabidopsis thaliana]	0,917	-;1;-	
gi15222551	44,4	PRK (PHOSPHORIBULOKINASE); ATP binding / phosphoribulokinase/ protein binding [Arabidopsis thaliana]	0,917	13;16;16	5,9
gi238480186	25,3	glycine-rich protein [Arabidopsis thaliana]	0,915	4;5;4	46,2
gi15238197	87,1	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]	0,914	21;19;17	6,2
gi15232971	40,4	dihydrodipicolinate synthase 1 (DHDPS1) (DHDPS) (DHPS1) [Arabidopsis thaliana]	0,913	2;-;-	
gi15228194	42,4	SBPASE (sedoheptulose-bisphosphatase); phosphoric ester hydrolase/ sedoheptulose-bisphosphatase [Arabidopsis thaliana]	0,913	14;12;9	4,7
gi15230897	85,4	glycosyl hydrolase family 3 protein [Arabidopsis thaliana]	0,913	9;8;7	20,5
gi18411901	29,1	GRF2 (GENERAL REGULATORY FACTOR 2); protein binding / protein phosphorylated amino acid binding [Arabidopsis thaliana]	0,912	1;1;-	4,8
gi110743820	36,4	putative chitinase [Arabidopsis thaliana]	0,912	9;7;5	18,9
gi18399660	42,9	fructose-bisphosphate aldolase, putative [Arabidopsis thaliana]	0,909	11;9;9	9,8
gi15232682	71,1	heat shock cognate 70 kDa protein 3 (HSC70-3) (HSP70-3) [Arabidopsis thaliana]	0,909	3;5;2	7,9
gi15219200	49,0	curculin-like (mannose-binding) lectin family protein [Arabidopsis thaliana]	0,909	16;21;14	18,5
gi15215642	60,8	AT5g56010/MDA7_5 [Arabidopsis thaliana]	0,907	6;2;2	28,1
gi18399423	30,5	protein phosphatase 2C, putative / PP2C, putative [Arabidopsis thaliana]	0,905	-;-;1	

gi18406066	54,7	PP5.2 (PROTEIN PHOSPHATASE 5.2); phosphoprotein phosphatase/ protein binding / protein serine/threonine phosphatase [Arabidopsis thaliana]	0,905	-;1;-	
gi1143388	40,7	class III ADH, glutathione-dependent formaldehyde dehydrogenase. [Arabidopsis thaliana]	0,904	5;6;2	11,0
gi3763925	30,8	putative clathrin binding protein (epsin) [Arabidopsis thaliana]	0,902	-;1;1	5,1
gi15222928	25,2	unknown protein [Arabidopsis thaliana]	0,900	1;-;-	
gi222424791	62,5	AT3G14067 [Arabidopsis thaliana]	0,900	-;1;1	15,2
gi4587532	47,8	Strong similarity to F19I3.2 gi|3033375 putative berberine bridge enzyme from Arabidopsis thaliana BAC gb|AC004238. This gene	0,899	-;-;1	
gi15233779	76,5	cpHsc70-1 (chloroplast heat shock protein 70-1); ATP binding [Arabidopsis thaliana]	0,898	2;-;-	
gi79327392	35,0	PMDH2 (peroxisomal NAD-malate dehydrogenase 2); malate dehydrogenase [Arabidopsis thaliana]	0,898	3;9;8	10,2
gi15485232	52,1	selenium binding protein [Arabidopsis thaliana]	0,897	1;-;-	
gi15232438	69,0	unknown protein [Arabidopsis thaliana]	0,897	-;1;1	25,1
gi14532772	84,6	putative methionine synthase [Arabidopsis thaliana]	0,896	11;11;4	24,7
gi15224351	41,6	OASB (O-ACETYLSERINE (THIOL) LYASE B); cysteine synthase [Arabidopsis thaliana]	0,895	1;3;1	15,5
gi15227863	29,6	ACL (ACETONE-CYANOHYDRIN LYASE); hydrolase/ hydrolase, acting on ester bonds / methyl indole-3-acetate esterase/ methyl jasmonate esterase/ methyl salicylate esterase [Arabidopsis thaliana]	0,894	-;1;-	
gi15228048	42,5	MAT3 (methionine adenosyltransferase 3); copper ion binding / methionine adenosyltransferase [Arabidopsis thaliana]	0,891	2;1;2	13,7
gi145323784	27,5	APX1 (ascorbate peroxidase 1); L-ascorbate peroxidase [Arabidopsis thaliana]	0,891	8;1;2	20,5
gi15231715	38,5	fructose-bisphosphate aldolase, putative [Arabidopsis thaliana]	0,891	4;3;2	3,5
gi15222978	73,7	calcineurin-like phosphoesterase family protein [Arabidopsis thaliana]	0,890	3;-;3	14,9
gi15010652	60,6	At1g09780/F21M12_17 [Arabidopsis thaliana]	0,890	4;6;5	7,7
gi7546402	47,8	Chain A, Structures Of Adenylosuccinate Synthetase From Triticum Aestivum And Arabidopsis Thaliana	0,890	5;4;2	5,1
gi15240901	20,3	ribulose bisphosphate carboxylase small chain 3B / RuBisCO small subunit 3B (RBCS-3B) (ATS3B) [Arabidopsis thaliana]	0,889	1;2;2	9,3
gi18403095	67,8	THFS (10-FORMYLTETRAHYDROFOLATE SYNTHETASE); ATP binding / copper ion binding / formate-tetrahydrofolate ligase [Arabidopsis thaliana]	0,889	1;-;-	
gi15224582	24,2	GSTF10 (HALIANA GLUTATHIONE S-TRANSFERASE PHI 10); copper ion binding / glutathione binding / glutathione transferase [Arabidopsis thaliana]	0,889	3;5;6	21,7
gi15234641	37,4	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	0,888	-;2;2	42,6
gi1022805	41,9	phosphoglycerate kinase [Arabidopsis thaliana]	0,888	1;1;3	24,4
gi25083482	99,1	putative aminopeptidase [Arabidopsis thaliana]	0,887	9;3;5	22,1
gi18402264	120,2	ATUBA1; ubiquitin activating enzyme/ ubiquitin-protein ligase [Arabidopsis thaliana]	0,886	1;-;-	
gi15236211	18,4	ROC5 (ROTAMASE CYCLOPHILIN 5); peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	0,886	8;5;5	13,5
gi13899069	15,7	Unknown protein [Arabidopsis thaliana]	0,886	2;3;2	14,5

gi4455323	97,2	aminopeptidase-like protein [Arabidopsis thaliana]	0,886	4;3;1	26,6
gi18420348	43,0	fructose-bisphosphate aldolase, putative [Arabidopsis thaliana]	0,885	28;25;17	12,9
gi15232230	43,3	SOX (SULFITE OXIDASE); sulfite oxidase [Arabidopsis thaliana]	0,885	1;1;-	1,8
gi332198195	32,2	putative D-isomer specific 2-hydroxyacid dehydrogenase [Arabidopsis thaliana]	0,885	1;-;1	13,1
gi21537387	18,3	unknown [Arabidopsis thaliana]	0,883	-;3;-	
gi18414298	47,5	ATMDAR2; monodehydroascorbate reductase (NADH) [Arabidopsis thaliana]	0,883	-;2;4	22,1
gi15239438	40,0	unknown protein [Arabidopsis thaliana]	0,882	2;6;4	6,9
gi3549670	46,6	putative protein [Arabidopsis thaliana]	0,880	-;3;1	1,8
gi30689934	19,7	APT1 (ADENINE PHOSPHORIBOSYL TRANSFERASE 1); adenine phosphoribosyltransferase [Arabidopsis thaliana]	0,878	3;3;-	15,1
gi15221116	31,9	ATGLX1 (GLYOXALASE I HOMOLOG); lactoylglutathione lyase/ metal ion binding [Arabidopsis thaliana]	0,877	9;7;6	4,7
gi15237018	25,7	NDPK3 (NUCLEOSIDE DIPHOSPHATE KINASE 3); ATP binding / nucleoside diphosphate kinase [Arabidopsis thaliana]	0,876	1;-;-	
gi6899921	51,9	putative protein [Arabidopsis thaliana]	0,876	2;-;-	
gi16648738	25,1	F25I18.1/F25I18.1 [Arabidopsis thaliana]	0,871	-;1;1	21,0
gi22531054	55,8	serine carboxypeptidase 1 precursor-like protein [Arabidopsis thaliana]	0,870	1;2;2	5,6
gi15240349	137,4	OXP1 (OXOPROLINASE 1); 5-oxoprolinase (ATP-hydrolyzing)/ hydrolase [Arabidopsis thaliana]	0,870	2;-;-	
gi30684106	49,5	UDP-glucoronosyl/UDP-glucosyl transferase family protein [Arabidopsis thaliana]	0,869	1;-;-	
gi18410809	54,1	glycoside hydrolase family 28 protein / polygalacturonase (pectinase) family protein [Arabidopsis thaliana]	0,868	-;1;1	30,0
gi15241849	71,3	HSC70-1 (HEAT SHOCK COGNATE PROTEIN 70-1); ATP binding [Arabidopsis thaliana]	0,868	14;15;9	4,0
gi15223226	63,4	phosphoglucomutase, cytoplasmic, putative / glucose phosphomutase, putative [Arabidopsis thaliana]	0,865	1;-;-	
gi79325213	30,8	oxidoreductase, zinc-binding dehydrogenase family protein [Arabidopsis thaliana]	0,861	1;1;-	0,0
gi15219721	35,5	malate dehydrogenase, cytosolic, putative [Arabidopsis thaliana]	0,860	8;6;3	2,9
gi15240689	92,6	alpha-N-acetylglucosaminidase family / NAGLU family [Arabidopsis thaliana]	0,860	1;1;-	26,5
gi15242717	37,8	ADK2 (ADENOSINE KINASE 2); adenosine kinase/ copper ion binding / kinase [Arabidopsis thaliana]	0,859	1;1;1	35,4
gi152149571	47,2	Chain A, Crystal Structure Of Ll-Diaminopimelate Aminotransferase From Arabidopsis Thaliana	0,858	-;2;-	
gi110740085	42,7	s-adenosylmethionine synthetase like protein [Arabidopsis thaliana]	0,856	-;2;2	24,3
gi15239049	39,6	unknown protein [Arabidopsis thaliana]	0,856	9;12;9	10,0
gi14334768	43,5	putative uroporphyrinogen decarboxylase [Arabidopsis thaliana]	0,853	3;4;3	14,9
gi5107821	61,3	Arabidopsis thaliana thioglucosidase (GB:X79195); Pfam PF00232, Score=702.5, E=1.9e-207, N=1	0,852	18;25;11	32,8
gi18396217	29,8	GRF7 (GENERAL REGULATORY FACTOR 7); protein binding / protein phosphorylated amino acid binding [Arabidopsis thaliana]	0,849	1;-;-	
gi18394416	17,2	ubiquitin-conjugating enzyme, putative [Arabidopsis thaliana]	0,848	-;-;1	
gi227202778	32,4	AT4G39330 [Arabidopsis thaliana]	0,846	6;5;5	12,8

gi15239741	35,6	cinnamyl-alcohol dehydrogenase, putative (CAD) [Arabidopsis thaliana]	0,844	-;1;-	
gi222424556	45,4	AT3G59970 [Arabidopsis thaliana]	0,844	3;7;3	9,9
gi30683408	39,5	4-methyl-5(b-hydroxyethyl)-thiazole monophosphate biosynthesis protein, putative [Arabidopsis thaliana]	0,843	-;1;1	23,4
gi51968542	52,1	prolyl carboxypeptidase like protein [Arabidopsis thaliana]	0,843	1;-;1	25,4
gi30698194	37,1	3-dehydroquinate synthase, putative [Arabidopsis thaliana]	0,842	-;2;-	
gi13430632	53,8	putative glutathione reductase [Arabidopsis thaliana]	0,842	4;3;2	11,8
gi15230110	30,0	hydrolase, alpha/beta fold family protein [Arabidopsis thaliana]	0,842	-;1;-	
gi681912	35,0	RNA-binding protein cp33 [Arabidopsis thaliana]	0,841	-;-;5	
gi15218438	37,3	fructose-1,6-bisphosphatase, putative / D-fructose-1,6-bisphosphate 1-phosphohydrolase, putative / FBPase, putative [Arabidopsis thaliana]	0,840	1;6;6	15,7
gi2511580	25,8	multicatalytic endopeptidase [Arabidopsis thaliana]	0,840	-;1;-	
gi15229349	29,3	ribose 5-phosphate isomerase-related [Arabidopsis thaliana]	0,840	6;5;4	7,9
gi15238559	47,4	GS2 (GLUTAMINE SYNTHETASE 2); glutamate-ammonia ligase [Arabidopsis thaliana]	0,839	15;13;10	23,9
gi15226489	35,0	mannose 6-phosphate reductase (NADPH-dependent), putative [Arabidopsis thaliana]	0,839	2;2;-	6,8
gi15228687	53,1	UDP-glucose 6-dehydrogenase, putative [Arabidopsis thaliana]	0,838	-;3;3	18,0
gi18402340	12,8	KIS (KIESEL); unfolded protein binding [Arabidopsis thaliana]	0,838	2;-;-	
gi18405887	18,4	unknown protein [Arabidopsis thaliana]	0,836	2;2;2	16,6
gi12324583	81,7	putative heat-shock protein; 41956-44878 [Arabidopsis thaliana]	0,834	1;-;-	
gi15239586	99,0	inosine-uridine preferring nucleoside hydrolase family protein [Arabidopsis thaliana]	0,833	1;-;-	
gi15231939	60,7	2,3-biphosphoglycerate-independent phosphoglycerate mutase, putative / phosphoglyceromutase, putative [Arabidopsis thaliana]	0,831	6;6;4	5,7
gi15239993	27,5	unknown protein [Arabidopsis thaliana]	0,829	3;2;-	14,0
gi5734779	105,1	cytosolic tRNA-Ala synthetase [Arabidopsis thaliana]	0,827	7;4;2	12,7
gi15240912	20,3	ribulose bisphosphate carboxylase small chain 1B / RuBisCO small subunit 1B (RBCS-1B) (ATS1B) [Arabidopsis thaliana]	0,826	-;2;-	
gi79329220	28,5	GRF3 (GENERAL REGULATORY FACTOR 3); ATP binding / protein binding / protein phosphorylated amino acid binding [Arabidopsis thaliana]	0,824	2;1;7	10,8
gi15224312	28,3	chitinase, putative [Arabidopsis thaliana]	0,824	4;1;-	0,3
gi332643314	64,7	dihydroxy-acid dehydratase [Arabidopsis thaliana]	0,824	7;5;2	12,5
gi79324895	29,4	GRF9 (GENERAL REGULATORY FACTOR 9); calcium ion binding / protein binding / protein phosphorylated amino acid binding [Arabidopsis thaliana]	0,823	3;2;4	10,1
gi79475768	95,3	PPDK (pyruvate orthophosphate dikinase); kinase/ pyruvate, phosphate dikinase [Arabidopsis thaliana]	0,823	1;-;-	
gi15228537	18,6	avirulence-responsive protein-related / avirulence induced gene (AIG) protein-related [Arabidopsis thaliana]	0,822	1;-;-	
gi11242	45,1	fructose-bisphosphatase [Arabidopsis thaliana]	0,817	8;11;11	12,8

gi15229062	55,5	AMP-dependent synthetase and ligase family protein [Arabidopsis thaliana]	0,812	21;23;12	13,1
gi899153	62,9	beta-fructofuranosidase [Arabidopsis thaliana]	0,810	1;5;5	29,0
gi15219412	42,1	PGK (PHOSPHOGLYCERATE KINASE); phosphoglycerate kinase [Arabidopsis thaliana]	0,809	1;2;2	23,9
gi15223138	41,1	OPR1; 12-oxophytodienoate reductase [Arabidopsis thaliana]	0,808	2;2;2	15,2
gi15237716	33,8	BTR1L (BINDING TO TOMV RNA 1L (LONG FORM)); nucleic acid binding / single-stranded RNA binding [Arabidopsis thaliana]	0,806	2;-;3	14,7
gi15229231	36,9	GAPC1 (GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE C SUBUNIT 1); glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)/ glyceraldehyde-3-phosphate dehydrogenase [Arabidopsis thaliana]	0,806	1;-;1	20,4
gi8885622	31,7	N-glyceraldehyde-2-phosphotransferase-like [Arabidopsis thaliana]	0,806	4;4;5	12,8
gi15238686	84,3	ATMS1; 5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase/ copper ion binding / methionine synthase [Arabidopsis thaliana]	0,805	11;13;10	11,8
gi15231718	24,7	peroxiredoxin type 2, putative [Arabidopsis thaliana]	0,805	-;-;4	
gi15219272	110,2	ATPPC1 (PHOSPHOENOLPYRUVATE CARBOXYLASE 1); catalytic/ phosphoenolpyruvate carboxylase [Arabidopsis thaliana]	0,804	1;-;-	
gi332197529	53,9	betaine aldehyde dehydrogenase 1 [Arabidopsis thaliana]	0,804	3;-;-	
gi145325451	21,0	pyrrolidone-carboxylate peptidase family protein [Arabidopsis thaliana]	0,803	-;-;1	
gi145334663	34,0	SOS4 (SALT OVERLY SENSITIVE 4); kinase/ pyridoxal kinase [Arabidopsis thaliana]	0,802	1;-;-	
gi18413869	27,1	binding / catalytic/ coenzyme binding [Arabidopsis thaliana]	0,802	11;8;16	8,4
gi3063661	17,0	nucleoside diphosphate kinase Ia [Arabidopsis thaliana]	0,801	1;1;1	7,7
gi21553827	48,9	polygalacturonase, putative [Arabidopsis thaliana]	0,800	2;2;2	18,5
gi18398038	35,7	kelch repeat-containing protein [Arabidopsis thaliana]	0,800	2;-;1	25,3
gi145360230	36,8	SNG1 (SINAPOYLGLUCOSE 1); serine-type carboxypeptidase/ sinapoylglucose-malate O-sinapoyltransferase [Arabidopsis thaliana]	0,799	6;6;3	2,4
gi15236591	34,6	aldose 1-epimerase family protein [Arabidopsis thaliana]	0,799	1;-;1	21,9
gi15222443	46,7	HEMB1; catalytic/ metal ion binding / porphobilinogen synthase [Arabidopsis thaliana]	0,799	4;4;2	11,2
gi21592545	77,2	glycyl tRNA synthetase, putative [Arabidopsis thaliana]	0,798	1;-;-	
gi15229497	40,3	(S)-2-hydroxy-acid oxidase, peroxisomal, putative / glycolate oxidase, putative / short chain alpha-hydroxy acid oxidase, putative [Arabidopsis thaliana]	0,792	1;2;1	25,6
gi22329686	26,0	unknown protein [Arabidopsis thaliana]	0,788	2;1;-	10,3
gi15240939	51,6	seryl-tRNA synthetase / serine--tRNA ligase [Arabidopsis thaliana]	0,788	1;-;-	
gi15220620	42,2	HPR; glycerate dehydrogenase/ poly(U) binding [Arabidopsis thaliana]	0,787	11;16;9	16,8
gi240254631	109,7	ATPPC2 (PHOSPHOENOLPYRUVATE CARBOXYLASE 2); catalytic/ phosphoenolpyruvate carboxylase [Arabidopsis thaliana]	0,785	3;-;4	25,8
gi79327622	27,7	GRF6 (G-box regulating factor 6); protein binding / protein phosphorylated amino acid binding [Arabidopsis thaliana]	0,784	1;3;-	31,6

gi15912233	57,9	At2g41680/T32G6.20 [Arabidopsis thaliana]	0,782	1;-;-	
gi79325249	21,1	FSD1 (FE SUPEROXIDE DISMUTASE 1); copper ion binding / superoxide dismutase [Arabidopsis thaliana]	0,781	-;2;-	
gi15236566	17,5	major latex protein-related / MLP-related [Arabidopsis thaliana]	0,779	13;9;10	20,8
gi15241945	42,7	GME (GDP-D-MANNOSE 3',5'-EPIMERASE); GDP-mannose 3,5-epimerase/ NAD or NADH binding / catalytic [Arabidopsis thaliana]	0,778	4;-;-	
gi6723413	44,6	branched-chain-amino-acid transaminase-like protein [Arabidopsis thaliana]	0,778	2;2;-	15,1
gi22326646	108,2	tudor domain-containing protein / nuclease family protein [Arabidopsis thaliana]	0,777	1;-;-	
gi15223126	20,8	lactoylglutathione lyase, putative / glyoxalase I, putative [Arabidopsis thaliana]	0,775	2;-;-	
gi15234763	28,4	defense-related protein, putative [Arabidopsis thaliana]	0,775	3;6;7	3,5
gi18410820	33,7	isoflavone reductase, putative [Arabidopsis thaliana]	0,774	1;2;2	8,7
gi79324923	29,4	unknown protein [Arabidopsis thaliana]	0,774	-;-;2	
gi13605559	51,7	AT3g03250/T17B22_6 [Arabidopsis thaliana]	0,769	3;5;3	3,0
gi15236570	21,4	disease resistance-responsive family protein / dirigent family protein [Arabidopsis thaliana]	0,769	2;1;2	16,8
gi15231702	46,5	monodehydroascorbate reductase, putative [Arabidopsis thaliana]	0,768	-;5;5	11,9
gi18401160	34,2	HEMD; uroporphyrinogen-III synthase [Arabidopsis thaliana]	0,768	-;-;2	
gi1321684	73,4	beta-fructosidase [Arabidopsis thaliana]	0,767	2;5;3	10,9
gi18391442	42,6	DET3 (DE-ETIOLATED 3); proton-transporting ATPase, rotational mechanism [Arabidopsis thaliana]	0,765	-;1;-	
gi7362755	50,0	beta-glucosidase-like protein [Arabidopsis thaliana]	0,764	-;1;-	
gi15221444	44,4	GTP binding [Arabidopsis thaliana]	0,763	2;1;1	11,1
gi19347816	67,0	putative poly(A)-binding protein [Arabidopsis thaliana]	0,763	-;-;1	
gi15218382	64,0	PATL1 (PATELLIN 1); transporter [Arabidopsis thaliana]	0,762	8;5;6	38,7
gi15233272	27,2	TPI (TRIOSEPHOSPHATE ISOMERASE); triose-phosphate isomerase [Arabidopsis thaliana]	0,761	11;8;12	9,7
gi18417127	33,1	unknown protein [Arabidopsis thaliana]	0,760	-;-;1	
gi2828296	68,0	RNase L inhibitor-like protein [Arabidopsis thaliana]	0,756	2;-;-	
gi15241839	48,2	unknown protein [Arabidopsis thaliana]	0,755	3;-;-	
gi15219722	46,4	aldo/keto reductase family protein [Arabidopsis thaliana]	0,753	1;3;5	29,8
gi134104574	33,6	Chain A, Crystal Structure Of O-Acetylserine Sulfhydrylase From Arabidopsis Thaliana In Complex With C-Terminal Peptide From Arabidopsis Serine Acetyltransferase	0,753	5;4;2	6,4
gi15226134	35,9	cinnamoyl-CoA reductase family [Arabidopsis thaliana]	0,751	-;1;-	
gi15229722	144,5	AAO2 (ALDEHYDE OXIDASE 2); aldehyde oxidase [Arabidopsis thaliana]	0,748	3;-;-	
gi62319055	19,7	adenosine kinase like protein [Arabidopsis thaliana]	0,745	2;3;3	20,0
gi42572441	38,2	HEME1; uroporphyrinogen decarboxylase [Arabidopsis thaliana]	0,742	2;5;-	1,4
gi186511795	21,6	acireductone dioxygenase [iron(II)-requiring]/ metal ion binding [Arabidopsis thaliana]	0,742	3;-;-	
gi15231805	44,0	ESM1 (epithiospecifier modifier 1); carboxylesterase/ hydrolase, acting on ester bonds [Arabidopsis thaliana]	0,740	-;3;1	3,2
gi18400986	22,7	unknown protein [Arabidopsis thaliana]	0,734	-;-;2	
gi15222310	25,6	SIRANBP; Ran GTPase binding [Arabidopsis thaliana]	0,731	8;15;14	8,4

gi15223825	23,6	DABB1 (DIMERIC A/B BARREL DOMAINS-PROTEIN 1) [Arabidopsis thaliana]	0,728	1;-;-	
gi18416304	79,0	peptidase M3 family protein / thimet oligopeptidase family protein [Arabidopsis thaliana]	0,728	-;1;-	
gi21555308	15,0	Photosystem I reaction center subunit IV B, chloroplast precursor (PSI-E B) [Arabidopsis thaliana]	0,726	-;1;1	41,3
gi15225896	27,7	PMM (PHOSPHOMANNOMUTASE); phosphomannomutase [Arabidopsis thaliana]	0,726	3;3;2	7,5
gi21537026	50,8	beta-N-acetylhexosaminidase-like protein [Arabidopsis thaliana]	0,720	-;1;1	25,9
gi79318240	77,0	ATSBT5.2; identical protein binding / serine-type endopeptidase [Arabidopsis thaliana]	0,719	1;-;-	
gi15226862	100,2	disease resistance family protein [Arabidopsis thaliana]	0,717	14;12;7	7,4
gi186509703	28,8	VTC4; 3'(2'),5'-bisphosphate nucleotidase/ L-galactose-1-phosphate phosphatase/ inositol or phosphatidylinositol phosphatase/ inositol-1(or 4)-monophosphatase [Arabidopsis thaliana]	0,717	2;4;3	9,6
gi186503279	27,1	TED4 (REVERSAL OF THE DET PHENOTYPE 4); heme oxygenase (decyclizing) [Arabidopsis thaliana]	0,716	-;-;2	
gi15228368	75,0	ASD1 (ALPHA-L-ARABINOFURANOSIDASE 1); alpha-N-arabinofuranosidase/ hydrolase, acting on glycosyl bonds / xylan 1,4-beta-xylosidase [Arabidopsis thaliana]	0,712	12;6;9	2,3
gi330253651	96,5	beta galactosidase 9 [Arabidopsis thaliana]	0,710	2;-;-	
gi145327759	40,8	phosphoribulokinase/uridine kinase-related [Arabidopsis thaliana]	0,709	-;3;-	
gi15227700	24,4	Ran-binding protein 1b (RanBP1b) [Arabidopsis thaliana]	0,709	4;6;3	10,3
gi227202864	20,9	AT1G07890 [Arabidopsis thaliana]	0,708	7;-;-	
gi15242870	55,0	PAP26 (PURPLE ACID PHOSPHATASE 26); acid phosphatase/ protein serine/threonine phosphatase [Arabidopsis thaliana]	0,707	1;-;-	
gi21593566	46,9	histidinol dehydrogenase [Arabidopsis thaliana]	0,704	1;-;-	
gi30690246	22,5	PYR6; cytidylate kinase/ uridylate kinase [Arabidopsis thaliana]	0,702	-;-;2	
gi15242099	35,4	aldose 1-epimerase family protein [Arabidopsis thaliana]	0,702	1;-;-	
gi2664210	63,6	asparaginyl-tRNA synthetase [Arabidopsis thaliana]	0,698	1;-;-	
gi42569818	109,7	DPE2 (DISPROPORTIONATING ENZYME 2); 4-alpha-glucanotransferase/ heteroglycan binding [Arabidopsis thaliana]	0,694	2;2;-	29,3
gi2654226	119,8	aminoacyl-t-RNA synthetase [Arabidopsis thaliana]	0,694	2;-;-	
gi227202764	14,1	AT1G09310 [Arabidopsis thaliana]	0,691	20;16;15	11,7
gi15232826	29,9	esterase/lipase/thioesterase family protein [Arabidopsis thaliana]	0,684	2;-;-	
gi118138607	38,1	Chain A, Crystal Structure Of Arabidopsis Thaliana Double Bond Reductase (At5g16970)-Apo Form	0,684	-;2;2	14,3
gi15237947	51,9	UTP--glucose-1-phosphate uridylyltransferase, putative / UDP-glucose pyrophosphorylase, putative / UGPase, putative [Arabidopsis thaliana]	0,684	1;2;-	9,7
gi15231303	28,7	ATEXLA1 (ARABIDOPSIS THALIANA EXPANSIN-LIKE A1) [Arabidopsis thaliana]	0,684	8;8;7	10,0
gi3759177	32,3	3-phosphoserine phosphatase [Arabidopsis thaliana]	0,682	-;-;2	
gi227206272	60,7	AT1G22530 [Arabidopsis thaliana]	0,676	2;-;-	
gi18411929	25,6	ATGSTU19 (GLUTATHIONE S-TRANSFERASE TAU 19); glutathione binding / glutathione transferase [Arabidopsis thaliana]	0,673	-;5;6	0,5

gi18418410	35,5	EMB1241 (embryo defective 1241); adenyl-nucleotide exchange factor/ chaperone binding / protein binding / protein homodimerization [Arabidopsis thaliana]	0,671	2;-;-	
gi15232963	17,8	dimethylmenaquinone methyltransferase family protein [Arabidopsis thaliana]	0,670	-;1;-	
gi15237183	60,7	OVA6 (OVULE ABORTION 6); ATP binding / aminoacyl-tRNA ligase/ nucleotide binding / proline-tRNA ligase [Arabidopsis thaliana]	0,669	1;-;-	
gi15218112	73,9	jacalin lectin family protein [Arabidopsis thaliana]	0,668	2;2;1	14,4
gi15218517	25,9	ATGSTU18 (GLUTATHIONE S-TRANSFERASE TAU 18); glutathione transferase [Arabidopsis thaliana]	0,663	-;1;-	
gi15223252	67,3	phosphoglucomutase, putative / glucose phosphomutase, putative [Arabidopsis thaliana]	0,642	1;-;1	7,7
gi15233990	39,5	oxidoreductase family protein [Arabidopsis thaliana]	0,621	1;-;-	
gi60594285	39,4	Chain A, X-Ray Structure Of Gene Product From Arabidopsis Thaliana At4g09670	0,621	1;-;-	
gi227206232	29,8	AT3G16420 [Arabidopsis thaliana]	0,620	-;3;-	
gi15240625	47,7	transaldolase, putative [Arabidopsis thaliana]	0,618	-;1;-	
gi222424902	85,4	AT1G17220 [Arabidopsis thaliana]	0,615	-;-;1	
gi14596185	43,7	similar to dihydroflavonol reductase [Arabidopsis thaliana]	0,613	-;3;-	
gi15226830	59,6	MEE23 (MATERNAL EFFECT EMBRYO ARREST 23); FAD binding / catalytic/ electron carrier/ oxidoreductase [Arabidopsis thaliana]	0,602	1;-;3	41,7
gi110740822	48,1	hypothetical protein [Arabidopsis thaliana]	0,596	-;2;4	13,0
gi15238541	33,4	unknown protein [Arabidopsis thaliana]	0,593	1;-;-	
gi15228027	37,5	ribose-phosphate pyrophosphokinase 4 / phosphoribosyl diphosphate synthetase 4 (PRS4) [Arabidopsis thaliana]	0,574	-;2;-	
gi18397283	129,9	CARB (CARBAMOYL PHOSPHATE SYNTHETASE B); ATP binding / carbamoyl-phosphate synthase/ catalytic [Arabidopsis thaliana]	0,508	1;-;-	
gi42567935	40,1	GDSL-motif lipase/hydrolase family protein [Arabidopsis thaliana]	**0,452**	-;1;-	
gi145336172	104,9	unknown protein [Arabidopsis thaliana]	**0,367**	1;-;-	
gi15229749	**77,5**	**copper amine oxidase, putative [Arabidopsis thaliana]**	**0,077**	**5;4;5**	**10,1**

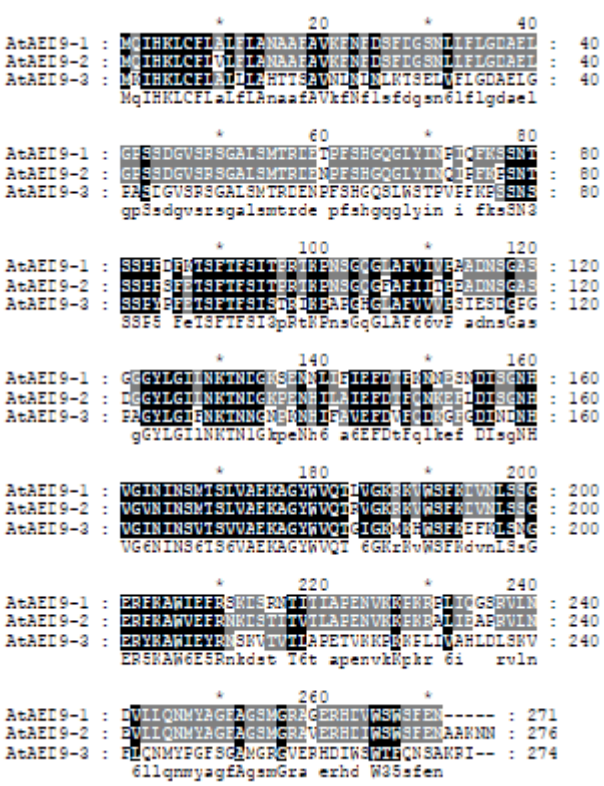


Figure 45: Alignment of the protein sequences of *At*AED9-1, *At*AED9-2 and *At*AED9-3.

This figure illustrates the sequence similarities between *At*AED9-1, *At*AED9-2 and *At*AED9-3.
Alignment is based on the protein sequence of the three proteins. The size/length differs between the proteins.
Identical sequences are highlighted in black, two matching amino acids out of three are marked in grey, whereas three differing amino acids are not highlighted. The alignment was done using the software GeneDoc, which is a full featured multiple sequence alignment editor:
http://www.nrbsc.org/gfx/genedoc/

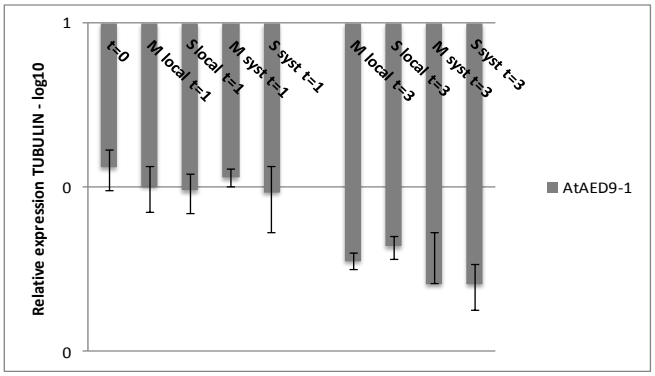


Figure 46: Expression of *AtAED9-1* in the *aed9-1* KO mutant during a SAR experiment, in which *Pst* AvrRpm1 was used

t=0 shows the expression without any treatment; M is the abbreviation for the MOCK-treated tissue; local=locally-treated tissue (1°); syst=systemic tissue (2°); t=1 is one day after infection; t=3 is three days after infection.
Same result was detectable during a SAR experiment, in which *Pst* AvrRps4 was used.

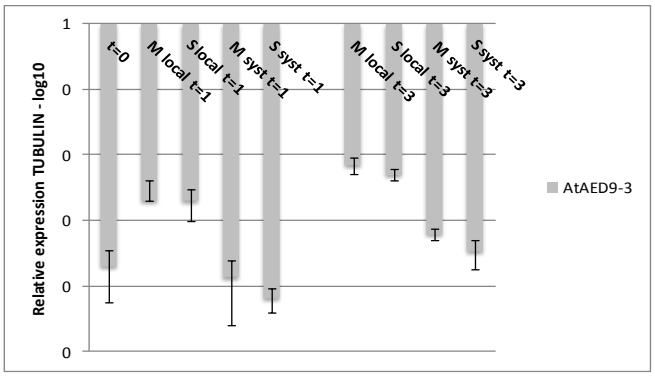


Figure 47: Expression of *AtAED9-3* in the *aed9-3* KO mutant during a SAR experiment, in which *Pst* AvrRpm1 was used.

t=0 shows the expression without any treatment; M is the abbreviation for the MOCK-treated tissue; local=locally-treated tissue (1°); syst=systemic tissue (2°); t=1 is one day after infection; t=3 is three days after infection.
Same result was detectable during a SAR experiment, in which *Pst* AvrRps4 was used.

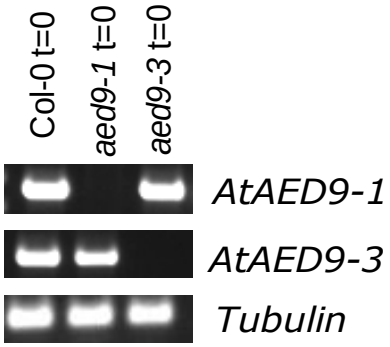


Figure 48: RT-PCR for AtAED9-1 and AtAED9-3 in untreated tissue (t=0) of Col-0, *aed9-1* and *aed9-3.*

AtAED9-1 is not detectable in *aed9-1*; *AtAED9-3* is not detectable in *aed9-3*. Tubulin functions as equal loading control.

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Poster und Vortrag

Heiko Breitenbach, Hakan Sarioglu, Thomas Colby, Lucia Jorda, Jane E. Parker, A. Corina Vlot "Proteins involved in systemic acquired resistance (SAR) in plants", International Conference on Arabidopsis Research (ICAR) 2012, Vienna, Austria, **Poster**

Heiko Breitenbach, Marion Wenig, Claudia Knappe, Hakan Sarioglu, Thomas Colby, Lucia Jorda, Jane E. Parker, A. Corina Vlot "Proteins involved in systemic acquired resistance (SAR) in plants", International Conference on Arabidopsis Research (ICAR) 2011, Madison, Wisconsin, USA, **Poster**

Heiko Breitenbach, Hakan Sarioglu, Thomas Colby, Lucia Jorda, Jane E. Parker, A. Corina Vlot "Proteic Signals Involved in Systemic Acquired Resistance (SAR) in Plants", International Conference on Proteomics in Plants, Microorganisms and Environment, Luxembourg City, Luxembourg, **Vortrag**