# Plant Defences

# SALICYLATE BIOSYNTHETIC GENES FROM *PSEUDOMONAS AERUGINOSA* USED TO MANIPULATE SALICYLATE PRODUCTION IN *ARABIDOPSIS THALIANA*

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Salicylate (SA) is a key signaling compound involved in the activation of certain plant defense responses (for a review see reference 1). Application of SA induces systemic acquired resistance (SAR), a mechanism by which the entire plant develops increased resistance to viral, bacterial and fungal pathogens. Furthermore, these plants respond to pathogen attack by increasing the levels of endogenous SA locally (at the site of infection) and systemically. The essential contribution of SA to SAR has been demonstrated in transgenic tobacco and Arabidopsis expressing a bacterial gene (*nahG*) for salicylate hydroxylase, which converts SA to catechol. *NahG* plants accumulate little or no SA and are defective in their ability to establish SAR. Genetic manipulation of SA biosynthesis in plants may open up new approaches to study the mechanisms involved in SAR and may also help to engineer plants with improved resistance to pathogens. Since SA biosynthetic genes from plants are not available yet, we have recruited these genes from the gram-negative bacterium *Pseudomonas aeruginosa*. In this bacterium, SA is the precursor of the siderophore pyochelin (Fig. 1), a secreted compound which complexes not only Fe(III), but also Zn(II), Co(II), and Ni(II) ions and delivers them to the cell (2). Most of the genes necessary for SA and pyochelin formation are clustered and organized in two operons in the *P. aeruginosa* chromosome (3, 4, 5). The *pchA* and *pchB* encoded enzymes convert chorismate to SA via isochorismate (3; C. Gaille, C. Reimmann & D. Haas, unpublished). SA is then activated by PchD and subsequently coupled to two molecules of cysteine involving the peptide synthetases PchE and PchF (4, 5, 6). Pyochelin formation requires also *pchG* (our unpublished results), and a thioesterase encoded by *pchC*, which probably helps to release the endproduct pyochelin from its thiol template (4).

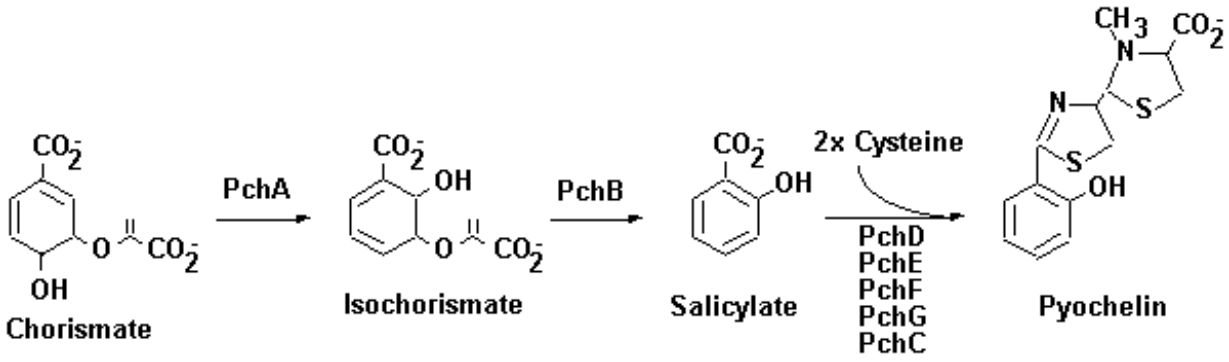


Fig.1 Biosynthetic pathway for salicylate and pyochelin in *P. aeruginosa*

PchA and PchB are the first SA biosynthetic enzymes described in molecular detail and it therefore seemed attractive to use the corresponding genes to engineer SA production in plants. To avoid separate expression of two genes in transgenic plants and thus formation of the potentially unstable intermediate isochorismate, we first created a gene fusion of *pchB* and *pchA* to combine the isochorismate synthase activity of PchA with the isochorismate lyase activity of PchB in a single protein. The activity of this novel salicylate synthase was tested by replacing the original *pchBA* genes in the chromosome of *P. aeruginosa* by the new *pchB-A* gene fusion. SA and pyochelin levels produced by mutant and wildtype strains were compared. The mutant strain produced 60% of the salicylate levels measured in the wildtype whereas production of pyochelin was similar in both strains.

In plants, chorismate is predominantly found in chloroplasts. Therefore, salicylate synthase (PchB-A fusion) was expressed under the control of the constitutive 35S-promoter in transgenic Arabidopsis plants with or without a plastid targeting signal. Plants expressing the cytoplasmic form of salicylate synthase showed a slight increase in free SA-levels and an up to eight-fold increase in the level of SA-conjugates, demonstrating that salicylate synthase can be expressed in an enzymatically active form in plants. These transgenic plants expressed defense related genes such as PR-1 and showed an increased disease resistance against infection by the fungal pathogen *Peronospora parasitica*. Transgenic plants expressing salicylate synthase targeted to the chloroplasts, however, showed a strongly dwarfed phenotype, indicating that due to high substrate availability, phytotoxic SA levels may have been reached.

We conclude from these results that it is possible to improve disease resistance in plants by moderately increasing SA levels through the expression of a bacterial salicylate synthase. SA overproducing plants may be useful to study SA-conjugate formation and transport, and to analyse the consequences of a constitutive expression of an otherwise inducible defense machinery.

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# DIFFERENTIAL GENE EXPRESSION IN RESPONSE TO MECHANICAL WOUNDING AND INSECT FEEDING IN ARABIDOPSIS: A CDNA MICROARRAY STUDY.

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Wounding is a continual threat to the survival of all organisms. Responses to wounding have been extensively studied in plants, which, in the wild, seldom escape some degree of damage due to environmental insults, such as wind, sand, hail and rain. An open wound caused by mechanical wounding is a potential site of infection for pathogens, thus defense gene expression at the wound site is a barrier against opportunistic microorganisms. Plants respond to mechanical wounding with the induction of numerous genes. The first identified wound-inducible defense proteins in plants include proteinase inhibitors I and II from potato and tomato. In *Arabidopsis thaliana,* many other genes have been shown to be induced by mechanical wounding. The expression of a large number of these genes is induced by treatment with jasmonic acid (JA) or by its precursor 12-oxo-phytodienoic acid (OPDA), and these compounds, members of the jasmonate family, are essential *in vivo* regulators of defense gene expression. Other signals and stimuli lead also to the expression of genes in wounded plant tissues, although the relative contribution of molecules such as ethylene, abscisic acid and electrical signals is still unclear. The importance of water stress/hydraulic pressure changes to gene expression during wounding has received still less attention .

A large proportion of multicellular eukaryotes eat plants and a particularly common source of injury to plants is insect herbivory. Inevitably, insect feeding causes wounding of the plant but little is known about how plants distinguish and respond to the very different threats posed by mechanical wounding and herbivory. Although reports show that some genes or proteins can be activated by both mechanical wounding and insect challenge, other observations have revealed responses which are induced specifically or activated more rapidly by insect damage. Differences have been observed at the level of gene expression. As in mechanical wounding where jasmonates play important roles in gene expression, the ability of plants to produce or perceive members of this family of regulators is essential for their defense against insects.

Using a cDNA microarray, we analyzed the timing, dynamics and regulation of the expression of 150 genes in mechanically wounded leaves of *Arabidopsis thaliana*. Temporal accumulation of a group of transcripts was correlated with the appearance of lipid-derived signals of the jasmonate family. The analysis of the jasmonate-insensitive *coi1-1* Arabidopsis mutant allowed the identification of a large number of COI1-dependent and COI1-independent wound-inducible genes. Water stress was found to contribute to the regulation of an unexpectedly important fraction of these genes. Dramatically different transcript profiles were obtained when Arabidopsis plants were damaged by feeding larvae of the small white butterfly (*Pieris rapae*). A major difference was the relative lack of water-stress induced gene expression during insect feeding. Results illustrate fundamental differences in responses to damage due to mechanical wounding and to damage due to insect feeding and help to link feeding strategy to molecular responses in the plant.

# SYRINGOLIN PROTECTS AND CURES WHEAT FROM POWDERY MILDEW

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In rice, acquired resistance towards *Pyricularia oryzae*, the fungal pathogen causing rice blast disease, can be triggered by inoculation with the non-host pathogen *Pseudomonas syringae* pv. *syringae* (PSS) (Smith and Métraux, 1991). One of the bacterial determinants that is recognized by rice plants is syringolin A, an elicitor that is secreted *in planta* and whose production is under the control of the *gacA/gacS* two-component regulatory system. (Reimmann *et al.*, 1995)

Syringolin A (MW 493 Da) is an unusual peptide consisting of a 12-membered ring formed by the two non-proteinogenic amino acids 5-methyl-4-amino-2-hexenoic acid and 3,4-dehydrolysine (Fig. 1). The a-amino group of the latter is connected by a peptide bond to a valine that in turn is linked to a second valine via a ureido group (Wäspi *et al.*, 1998). Syringolin A is the major component of a family of related compounds with very similar biological activities (syringolin B to F; Wäspi *et al.*, 1999).

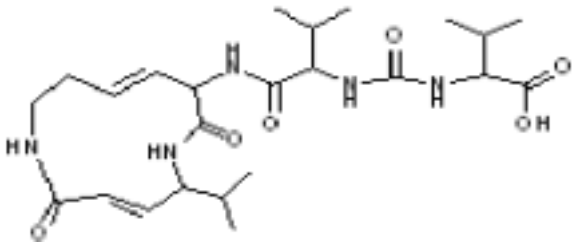

**Fig.1**: Structure of syringolin A, the major variant of a family of similar compounds (syringolin B to F) that differ from syringolin A by the substitution of valine(s) by isoleucine(s), of dehydrolysine by lysine, and by combinations thereof (Wäspi *et al.*, 1999)

Application of syringolin A (20 ppm solution) onto rice leaves leads to the accumulation of defense-related transcripts (*Rir1a, Pir2, Pir3,* and *Pir7b*), and to enhanced resistance against *P. oryzae* This resistance apparently is not due to a direct fungitoxic effect of syringolin A to *P. oryzae* because no such effect could be observed with high syringolin concentrations on this and other fungi *in vitro*.

Syringolin A (spray application of a 20 ppm solution) a has also a protective and an even more dramatic curative effect against powdery mildew on wheat that resembles a hypersensitive response Because powdery mildew as an obligate biotroph cannot be grown *in vitro*, we cannot exclude that syringolin A is directly toxic to this fungus. However, it does not inhibit the germination rate *in vitro* Furthermore, syringolin A induces accumulation of a Pir7b protein homolog and other unknown proteins as visualized by 2-D gel separation of *in vitro* translation products in uninfected wheat, indicating that the compound activates host genes and that wheat can perceive the compound. However, in contrast to rice, the wheat *Wir1, Wir2, and Wir3* transcripts (the homologs of the rice *Rir1, Pir2,* and *Pir3* mRNAs) do not accumulate upon syringolin treatment. The *Wir* transcripts accumulate transiently upon powdery mildew inoculation, but vanish during the course of the infection Interestingly, some *Wir* genes are reactivated by syringolin treatment of infected wheat plants, suggesting that syringolin either reactivates successful defense responses, or inactivates a speculative mechanism in the pathogen that suppresses host defenses.

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# INTERACTIONS OF THE PLANT PARARETROVIRUS VIRION-ASSOCIATED PROTEINS WITH VIRUS AND HOST PROTEINS.

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Rice tungro bacilliform virus (RTBV) and Cauliflower mosaic virus (CaMV) are pararetroviruses which belong to the Caulimoviridae family [1]. Their genome consists of a circular double-stranded DNA molecule, of about 8 kbp, encapsidated as bacilliform (RTBV) or icosahedral (CaMV) virions. In addition to the capsid protein (CP) and to the proteins involved in replication, movement, transmission and regulation of viral gene expression, they also code for a small protein (12-15 kDa) which is found closely associated with the viral particles (virion-associated protein, VAP). This protein possesses a non-specific nucleic acid binding activity, and recently CaMV VAP was shown to be involved in virus aphid transmission [2]. However, the crucial function of VAP during the plant infection process is still unknown. To investigate the role(s) of pararetroviral VAPs, we are currently studying the interactions between these proteins and (1) the other viral proteins or (2) cellular proteins, and (3) their subcellular localization.

(1) RTBV VAP was shown to interact with the CP both in the yeast two-hybrid system and in vitro [3]. Analysis by mutagenesis allowed the characterization of the VAP-CP interaction. The minimal domain of the RTBV VAP required for the CP binding corresponds to the C-terminal region which also supports the nucleic acid binding activity. Three types of residues (prolines and hydrophobic and basic amino acids) play a key role in establishing the interaction. The VAP-interacting region of the CP is located just in front of the basic region and the Zn-finger motif, which are most likely to be involved in the recognition of the RTBV pregenomic RNA during the encapsidation process. This domain contains several crucial hydrophobic residues. Similar results were also obtained with the CaMV VAP. The nature of the crucial residues identified in our experiments suggest that the VAP-CP association results from hydrophobic interactions which could be stabilized by ionic links.

We have found that both CaMV and RTBV VAPs can form tetramers in parallel orientation in vitro [4]. The tetramerization occurs also in vivo. A motif located within the N-terminal region exhibits the heptad periodicity characteristic of coiled-coil structures and is involved in VAP oligomerization.

(2) CaMV VAP has been used as bait to screen an A. thaliana cDNA library with the yeast two-hybrid system. Several candidates, including a domain of a kinesin-like motor protein, have been isolated and their characterization is in progress.

(3) In order to study their subcellular localization, the ORFs corresponding to the CaMV and RTBV VAPs have been cloned fused to the Green Fluorescent Protein (GFP) gene in a transient expression vector. First results, from Nicotiana plumbaginifolia protoplast transfections, show that RTBV VAP-GFP fusion protein accumulates in the nucleus and associates with filaments in the cytoplasm. We are currently trying to identify the nature of these filaments.

Based on our present knowledge, the possible different function(s) of the pararetroviral VAPs in the viral infection cycle will be discussed.

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# GENETIC ANALYSIS OF NON-HOST RESISTANCE IN *HORDEUM VULGARE-PUCCINIA RECONDITA SP. TRITICI* INTERACTION

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Plants are non hosts for the majority of potential pathogens. In plant-fungus interactions, two levels of specificity have to be considered (Figure 1). The first level defines host (plant) species specificity. Here, compatibility is specific and resistance (non host resistance) is probably genetically complex. Once the basic compatibility has been established a second level of interaction concerns cultivar specificity. At this level, the resistance is specific and is controlled by gene for gene interactions. This second level has been extensively studied in the last 10 years with the isolation of a number of plant disease resistance genes.

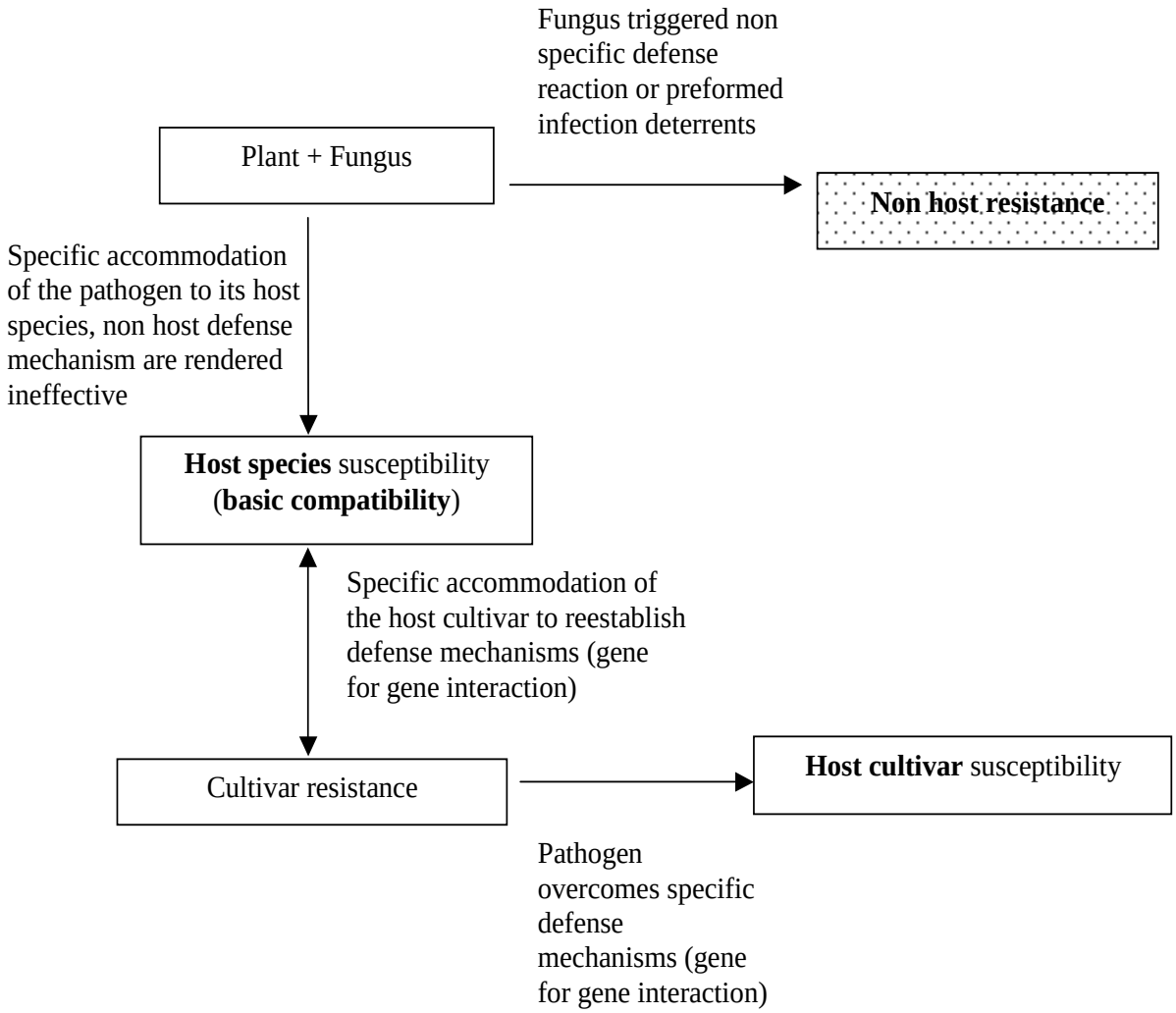


Figure 1: Postulated events leading to species and cultivar specificity of a fungal pathogen (Heath, 1981)

Mechanisms leading to non host resistance are poorly understood. Until now it has been impossible to study it at the genetic level since per definition there are no susceptible cultivars.

The distinction between host and non host is not always clear cut and some host-pathogen systems show a sort of "leaky" behavior making it difficult to give an absolute definition of a non host. Indeed it has been reported that the wheat leaf rust fungus *Puccinia recondita* sp. *tritici* can in very few cases infect barley cultivars and induce some sporulation. We believe that the close evolutionary relationship between the hosts (wheat and barley) and the pathogen (*Puccinia*) represents a good system to study mechanisms involved in non-host resistance at the genetic level.

We have observed a strong sporulation of wheat leaf rust on a barley cultivar (Bowman) after artificial infection. Although we found more chlorosis, the level of infection was comparable to the control susceptible wheat cultivar and reached a level never reported so far. Barley cultivars containing different leaf rust resistance genes (*Rph*) against barley leaf rust (*Puccinia hordei*) showed a resistant reaction to the wheat leaf rust. In order to determine if this resistance was due to the presence of the *Rph* genes and would therefore involve gene for gene interaction, we have infected near isogenic lines containing the same *Rph* resistance genes in the background of Bowman. All the lines showed a susceptible reaction comparable to Bowman after artificial infection with wheat leaf rust. This suggests that (i) the resistant reaction observed in the *Rph* containing cultivars is not due to a specific gene for gene interaction but is a classical non host resistance and (ii) a factor involved in non host resistance must be affected in the Bowman cultivar. To study the genetic inheritance of this factor we have crossed Bowman with a non host resistant cultivar (Cebada Capa) and studied the segregation of the resistance phenotype in the $F_2$ progeny. A 3:1 segregation was found indicating a dominant inheritance of non host resistance and suggesting that the factor affected in Bowman is a single recessive factor. We are currently mapping this factor and studying further the range of susceptibility to non host pathogens of Bowman.

# Poster P1

## TOWARDS THE DETERMINATION OF THE MOLECULAR BASIS OF BABA-MEDIATED POTENTIATION OF PLANT DEFENSES

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Non-protein amino acids such as gamma- or beta-amino-butyric acids (GABA and BABA, respectively) have known biological effects in animals and plants. In animals, GABA, like glycine, is a major inhibitory neurotransmitter and BABA is a partial agonist of the glycine receptor. In plants, GABA is produced as a response to stress and treatment with BABA was shown to protect against various pathogens.

The effect of BABA and its closely related isomers such as alpha-amino-butyric acid (AABA) and GABA was tested on the resistance of Arabidopsis thaliana against the oomycete pathogen *Peronospora parasitica*. The plants displayed a remarkable selectivity towards amino-butyric acid isomers and BABA was the only active structure showing a protective effect against *P. parasitica*. No direct antibiotic effect was observed against a broad range of fungi and bacteria and the protective effect of BABA is likely to result from an activation of disease resistance mechanisms in the host plant..

We have demonstrated that BABA acts independently of well known signaling cascades such as those involving salicylic acid (SA), jasmonic acid (JA) or ethylene (Eth). The study of the mode of action of BABA unveils a hitherto unknown hierarchy in the defence responses of plants against pathogens. In fact, BABA induces effective defence to an oomycete pathogen that is based on efficient and timely production of cell wall deposits (papillae) at the site of infection in absence of pathogenesis-related proteins (Prs).

BABA is also able to protect Arabidopsis against bacterial infection by *Pseudomonas syringae*. Since in this case, papilla formation is rather unlikely to be the reason for the protective effect, we also tested the above-mentioned signal transduction mutants. Interestingly, NahG plants and npr1 mutants were not protected by BABA suggesting that in this case the protection depends on the SA induction of PR-genes. Since the induction of PRs usually takes place at the site of pathogen infection even in a compatible interaction, albeit less intensively, we tested whether BABA might condition the plant to a faster PR response after pathogen attack. Indeed, BABA treatment can effectively condition the plants to induce PR-1 more rapidly and the response is similar to that observed in an interaction with avirulent forms of *P. syringae*. Similarly, this potentiation phenomenon by BABA operates also when abiotic stresses, like heatshock, are involved.

In summary, BABA has a distinct effect as a conditioner of plant defense responses and it acts at a very early step in the interaction between the plant and the pathogen. These experiments add to our understanding of the importance of induced defence responses in plants. The site of action of

BABA clearly represents an attractive target for the development of novel crop protectants which capitalize on the natural potential of plants to ward off pathogens.

In order to identify the genes involved in these potentiation events we screened T-DNA insertion mutant lines of *Arabidopsis thaliana* for non-inducibility by BABA. The phenotypic and molecular characterization of the resulting mutants will be presented.

# Poster P2

## THE COMPATIBLE INTERACTION BETWEEN POTATO AND *PHYTOPHTHORA INFESTANS*

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Aims and their significance
*Phytophthora* infestans causes late blight disease on potato and tomato. It is notorious for causing the Irish Potato Famine shortly after it first appeared in Europe in the 1840s. Today it is the most important disease of potato worldwide and more chemicals are applied per ha. to potato than to any other crop plant, mainly to combat late blight.
Despite intense research into the physiology of *Phytophthora* and its interaction with potato, we still know little about the molecular processes involved in infection and resistance. Since genetic techniques and tools for *P. infestans* are in their infancy, we are examining its interaction with potato using a genomics approach. We are looking at the genes induced in both host and pathogen during infection to learn more about the interaction. The information we acquire about the specific, *P. infestans*-potato interaction may also tell us more about the disease processes of other Oomycete plant pathogens. This information could be valuable in the fight against pathogens belonging to this family, which, although they continue to be referred to as "fungi" for convenience, belong to the Chromista and are only very distantly related to the true fungi, the Ascomycetes and Basidiomycetes.
In contrast to many other studies, we are concentrating on the compatible interaction. The induced genes are interesting at both the scientific and the biotechnological level. The plant genes may include classic PR genes; genes involved in late-expressed resistance, e.g. in limitation of lesion size and tolerance of the pathogen; and genes that are expressed in the compatible but not in the incompatible interaction. The latter genes will be particularly interesting as they may be involved in the establishment of the symbiotic relationship. At the biotechnological level the genes or their promoters may be useful in developing new resistance strategies.
The *P. infestans* genes induced during a compatible infection could tell us more about the interaction at the molecular level and may include pathogenicity or virulence factors.
Differential display
The first approach we used to find these genes was mRNA differential display. Detached leaves from axenically grown plants were inoculated with spores, and analysed over the first four hours after infection. During this time the first epidermal cell is penetrated and the first plant reactions can be detected. Half of the clones derived from differential display were of "fungal" origin, but only one, which was independently isolated three times, was shown to be induced. Two of the plant genes are induced by infection in intact plants. One of these genes is induced by *P. infestans* infection but only minimally by wounding. We are now looking for upstream regulatory elements in this gene that may be used to drive the expression of defence constructs in potato. The second differential display derived gene is induced rapidly by wounding as well as by infection.
Suppression Subtractive Hybridization

Differential display is a very inefficient technique so we used suppression subtractive hybridization (SSH) to screen for more genes induced during the interaction. SSH uses differential hybridization and selective PCR amplification and yields a normalized library of possibly induced genes. Unlike differential display, only two RNA samples can be analysed by SSH, the control and tester, but it is much more efficient and identifies genes with a wide range of expression levels.

We performed SSH with a number of different control and tester RNA populations; e.g. 0 hours post infection (p.i.) as control against both 5 and 48 hours p.i. as tester. In a preliminary test of which clones were indeed induced during infection, the cDNA inserts of 300 clones from each library were arrayed and analysed by inverse Northern; i.e. duplicate blots of the DNAs were made and hybridized with the original control and tester cDNA. Around 50% of the clones could be detected on inverse Northern blots and up to 35% of these gave a differential signal between control and tester probe that was clearly visible by eye. We have now examined 18 of these clones by Northern blot or RT-PCR: 15 of them are induced during compatible interactions between potato and *P. infestans*. Most of the clones in the libraries are from potato, and we screened for *P. infestans* clones in the libraries using labelled *P. infestans* genomic DNA.

Screening of the libraries is still in progress, but we have already isolated a number of potato genes that have not been described as pathogen induced before. The fungal clones isolated so far have no similarity to any of the *Phytophthora* ESTs in the database or in Genbank. But this is not surprising as there is still relatively little sequence information from the Oomycetes or indeed from any of the Chromista.

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# Poster P3

## WHAT WE LEARNED FROM SALICYLIC ACID INDUCTION-DEFICIENT MUTANTS OF *ARABIDOPSIS*

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In *Arabidopsis*, systemic acquired resistance against pathogens has been associated with the accumulation of salicylic acid (SA) and the expression of the proteins PR-1, PR-2 and PR-5. We isolated of two non-allelic mutants that do not accumulate SA after pathogen inoculation (Figure 1) because of a blockage in the pathway leading to salicylic acid biosynthesis.

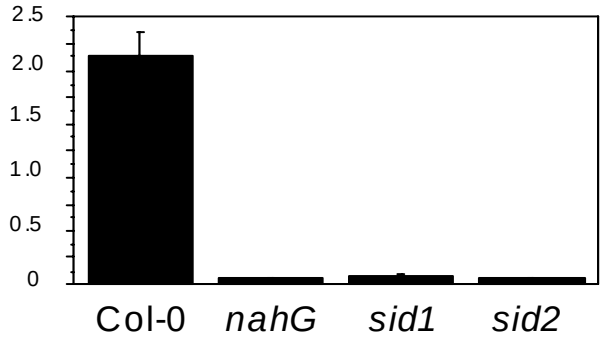


Figure 1. Accumulation of free SA 2 days after inoculation with *Pst* DC3000 carrying *avr Rpt2* at OD 0.2.

These salicylic acid induction-deficient (*sid*) mutants are more susceptible to virulent and avirulent forms of *Pseudomonas syringae* and *Peronospora parasitica*. However, *sid* mutants are not as susceptible to these pathogens as transgenic plants expressing the *nahG* gene, encoding a SA hydroxylase, which degrades SA to catechol (Table 1).

In contrast to *nahG* plants, the expression of PR-1 is strongly reduced, whereas PR-2 and PR-5 are still expressed after pathogen attack. Furthermore, the accumulation of the phytoalexin camalexin is normal (Figure 2).

| | Mock | *Pst/avrRpt2* |
|---|---|---|
| Col | 41% | 23% |
| *nahG* | 94% | 95% |
| *sid1* | 85% | 75% |
| *sid2* | 80% | 69% |

Table 1. Leaves showing sporulation of *P. parasitica* isolate NOCO 8 days after challenge infection of mock inoculated plants or of plants 5 days after pre-inoculation with *Pst* DC3000 carrying *avrRpt2*.

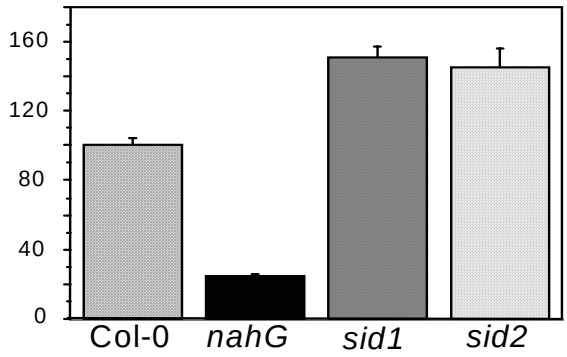


Figure 2. Camalexin accumulation 2 days after inoculation with *Pst* DC3000 carrying *avr Rpt2* at OD 0.2.

These results indicate that in *sid* mutants SA-independent compensation pathways are active that do not operate in *nahG* plants (Figure 3). One of the mutants is allelic to *eds5*, while the other mutant is novel.

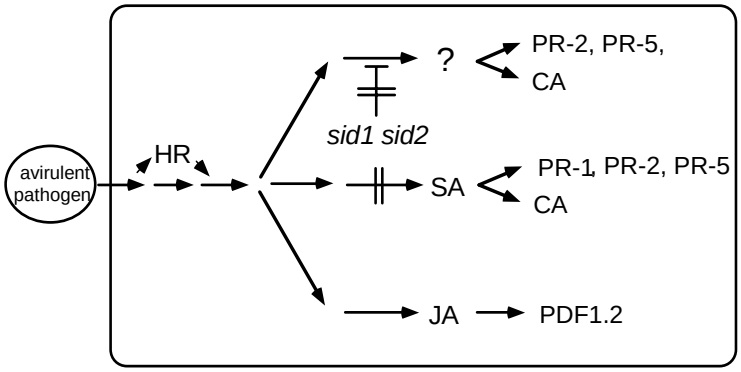


Figure 3. Model for the action of SID proteins in *Arabidopsis*.

References:

Nawrath C and Métraux J-P (1999). Salicylic acid induction-deficient mutants of Arabidopsis express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen infection. Plant Cell 11: 1393-1404.

# Poster P4

## ANTI-FUNGAL ACTIVITY OF A VIRALLY ENCODED GENE IN TRANSGENIC SWISS WHEAT VARIETIES.

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Key words: *Triticum aestivum* L., anti-fungal activity, *Tilletia tritici*, transformation, KP4

Abstract:

The cDNA encoding the antifungal protein KP4 from *Ustilago maydis*-infecting virus was inserted behind the ubiquitin promoter of maize and genetically transferred to wheat varieties particularly susceptible to stinking smut (*Tilletia tritici*) disease. The transgene was integrated and inherited over several generations. Of seven transgenic lines, three showed anti-fungal activity against *U. maydis*. The antifungal activity correlated with the presence of the KP4 transgene. KP4 transgenic, soil grown wheat plants exhibit increased endogenous resistance against stinking smut.

# Poster P5

## ASSEMBLY INTO FILAMENTS OF THE OGA-BINDING PROTEIN REMORIN

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Oligogalacturonides (OGAs), released from cell walls during pathogenesis, function as elicitors, triggering a variety of plant defense reactions. A search for OGA-binding proteins in plants revealed an OGA-binding phosphoprotein, remorin (1), a protein potentially involved in OGA-based signal transduction. Although strongly associated with plasma membranes, the protein does not have the structure of a typical membrane-bound receptor protein: instead, remorins are small, hydrophilic proteins. One strategy we are using to elucidate the role of the remorin gene family in plant defense reactions is a study of the structure of the recombinant protein. Remorin can be expressed at high levels in *E. coli* and can be easily purified in quantities sufficient for structural studies when fused to a cleavable histidine tag. As confirmed by circular dichroism analysis, the remorin C-terminal half forms a coiled coil structure. Negative staining electron microscopy techniques were used to show that the purified protein forms filamentous structures *in vitro*. Remorin-like proteins are encoded by large gene families in many plants, and the proteins appear to be plant-specific. Investigation of the subcellular localization of the protein is in progress.

1. Reymond P et al., Plant Cell 8:2265, 1996