

## Summary

Plants are sessile organisms that cannot flee from unfavorable conditions. Abiotic conditions, such as drought, cold, UV-irradiation, or flooding will severely influence plant fitness. In addition, plants can be attacked by a multitude of invaders, i.e. herbivorous insects or microbial pathogens. In order to cope with these threats plants have evolved sophisticated defensive mechanisms that limit damage caused by biotic or abiotic stress. In the work described in this thesis, we used the model plant species *Arabidopsis thaliana* to investigate molecular mechanisms involved in the ever ongoing battle between plants and their microbial and herbivorous enemies. In order to rapidly respond to pathogen or insect attack, plants possess a variety of inducible defense responses, which are initiated upon recognition of the attacker. The plant signaling molecules, salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) have been shown to play an important regulatory role in these responses. Their levels increase in invaded tissue and trigger defense reactions that mount resistance at the site of interaction (local) or throughout the whole plant (systemic). Although there are exceptions, resistance against biotrophic microbial pathogens is largely dependent on the action of SA, while JA-dependent defense responses are often effective against necrotrophic pathogens and insects. The plant hormone ET has a modulating role in both SA- and JA-dependent defense responses, but by itself can also confer resistance to some invaders. Recent advances in defense signaling research revealed that SA-, JA-, or ET-dependent defense responses do act independently of each other, but interact in a more complex signal-transduction network. For instance, induction of SA-dependent responses is known to suppress JA-dependent responses. This so-called cross-talk between signal transduction cascades is thought to provide the plant with a powerful regulatory potential, which helps the plant to “decide” which defensive strategy to follow, depending on the type of attacker it is encountering. Yet, it may also allow attackers to manipulate plants for their own benefit by shutting down induced defenses through influences on the signaling network.

In order to investigate to what extent plants make use of the interplay between SA-, JA-, and ET-dependent defense signaling pathways, we first compared the response of *Arabidopsis* to attack by different microbial pathogens and herbivorous insects, i.e. the bacterial pathogen *Pseudomonas syringae* pv. *tomato*, the fungal pathogen *Alternaria brassicicola*, tissue-chewing larvae of the cabbage butterfly (*Pieris rapae*), cell-piercing thrips (*Frankliniella occidentalis*), and phloem-sucking aphids (*Myzus persicae*). We monitored the levels of SA, JA, and ET over time for each *Arabidopsis*-attacker combination (typically 0–72 hr). Data in chapter 2 show that each *Arabidopsis*-attacker combination leads to the accumulation of a specific blend of these signaling molecules, called the “signal signature”. The signal signature of each interaction varied greatly in composition, amplitude, and timing, indicating that plants are highly flexible in their response to different invaders. In addition, the expression of all ~25,000 *Arabidopsis* genes was studied in response to each of the five attackers by using Affymetrix *Arabidopsis* whole-genome GeneChips. Analysis of global gene expression profiles demonstrated that the signal signature characteristic of each *Arabidopsis*-attacker combination is orchestrated into a surprisingly complex set of transcriptional alterations in which, in all cases, stress-related genes are over-represented. Comparison of the transcript profiles revealed that consistent changes induced by pathogens and insects with very different modes of attack can show considerable overlap. However, the majority of the induced changes in gene expression were attacker-specific. Notably, although *P. syringae*, *A. brassicicola*, *P. rapae* and *F. occidentalis* all stimulated JA biosynthesis and JA-responsive gene expression, the majority of the changes in JA-responsive gene expression were attacker-specific. Hence, defense signals such as JA play a primary role in the orchestration of the plant’s defense response, but other regulatory mechanisms, such as pathway cross-talk and additional attacker-induced signals, eventually shape the highly complex attacker-specific defense response.

Next, we investigated whether prior attack by one invader would influence the resistance against another attacker. To this end, we developed a bioassay in which plants were infested by JA-inducing larvae of the herbivore *P. rapae* and subsequently inoculated with various microbial pathogens. We hypothesized that herbivore feeding would lead to increased levels of JA and, thus, would induce resistance against microbial pathogens that are restricted by JA-dependent defense responses (e.g. *A. brassicicola*), while microbial pathogens arrested through SA-dependent defenses (e.g. turnip crinkle virus (TCV)) would not be affected. Larvae of *P. rapae* stimulated the production of JA and triggered a defense response that affected insect performance on systemic tissues. Although *A. brassicicola* is sensitive to JA-dependent defenses, herbivore-induced resistance was not effective against this

pathogen. To investigate the reason why *P. rapae*-induced defense was not effective against *A. brassicicola*, we analyzed the expression of *PDF1.2*, a JA-responsive marker gene for resistance to *A. brassicicola*. *PDF1.2* was activated upon mechanical damage but suppressed when wounding was inflicted by *P. rapae* feeding. Application of larval regurgitant to artificially wounded sites suppressed wound-induced *PDF1.2* expression as well, indicating that elicitors from *P. rapae* antagonize this JA-dependent defense response. This may explain the ineffectiveness of herbivore-induced resistance against *A. brassicicola*. Resistance against the biotrophic turnip crinkle virus (TCV) requires SA, but not JA and ET. Nevertheless, herbivore feeding strongly reduced TCV multiplication and TCV lesion formation, also in systemic tissues. Wounding alone was not effective, but application of regurgitant onto the wounds induced a similar level of protection. Analysis of SA-induced *PR-1* expression revealed that *P. rapae* feeding primes Arabidopsis leaves for augmented expression of SA-dependent defenses. Pharmacological experiments showed that ET acts synergistically on SA-induced *PR-1*, suggesting that the increased production of ET upon herbivore feeding sensitizes the tissue to respond faster to SA, thereby contributing to an enhanced defensive capacity towards pathogens, such as TCV, that trigger SA-dependent defenses upon infection. Hence, feeding by *P. rapae* triggers a surprisingly complex defense response that includes both synergistic and antagonistic effects on cross-talk between different signaling pathways that lead to resistance against microbial pathogens.

The observation that factors in the regurgitant of *P. rapae* suppress wound-induced expression of *PDF1.2* prompted us to investigate the molecular mechanism underlying this phenomenon. To investigate the mechanism by which *P. rapae* feeding suppresses *PDF1.2* expression, we studied the role of SA and abscisic acid (ABA), both of which have been implicated in antagonizing the JA-induced expression of *PDF1.2*. *P. rapae*-mediated suppression of *PDF1.2* was shown to be independent of SA for two reasons. Firstly, other JA-responsive genes, which were shown previously to be suppressed by SA were not affected by *P. rapae* feeding. Secondly, the regulatory protein NPR1, which is important in SA-mediated suppression of *PDF1.2*, is not required for *P. rapae*-mediated suppression of *PDF1.2*. However, the ABA biosynthesis mutant *aba2-1* showed a significantly increased *PDF1.2* expression upon feeding by *P. rapae*. Previously, ABA was shown to be an important regulator of AtMYC2, a transcription factor that activates specific JA-responsive genes (e.g. *VSP2* and *LOX2*), while suppressing other JA-responsive genes (e.g. *PDF1.2*). AtMYC2 was up-regulated in response to *P. rapae* feeding, but not upon mechanical damage. Like *aba2-1*, the AtMYC2 mutant *jin1-2* was also impaired in *P. rapae*-induced suppression of *PDF1.2* and showed high levels of *PDF1.2* expression upon insect feeding. Suppression of other wound-responsive, *P.*

*rapae*-suppressed genes, e.g. *ETHYLENE-RESPONSE FACTOR*, showed a strong *P. rapae*-induced expression pattern in *jin1-2*. Taken together, our results indicate that AtMYC2 is an important regulator of *P. rapae*-induced suppression of a specific branch of the JA-dependent host defense response. It is tempting to speculate that the specialist caterpillar, *P. rapae*, actively interferes with the host defense response and thereby makes it more suitable for infestation. On the other hand, the host might shut down unnecessary defense responses that do not contribute to the defense against this particular invader, in order to prioritize defenses that do affect caterpillar feeding.

Finally, the involvement of another transcription, AtMYB102, in resistance against *P. rapae* was investigated. This member of the MYB transcription factor family was previously shown to be induced upon wounding and osmotic stress. As both stresses occur during caterpillar attack, we hypothesized that AtMYB102 would also be involved in resistance against feeding *P. rapae* larvae. Indeed, independent experiments showed that *AtMYB102* is up-regulated upon caterpillar feeding. Histochemical analysis of an *AtMYB102*:*GUS* reporter line indicated that *AtMYB102* was expressed along the edges of the feeding sites. Knockout *myb102* mutants with a T-DNA insertion in the *AtMYB102* gene allowed the caterpillars to gain a 1.5-fold higher weight than caterpillars feeding from Col-0 wild-type plants. Moreover, approximately 50% of all larvae feeding on *myb102* plants had pupated within 14 days, whereas only 5% did on wild-type plants. These results indicate that MYB102 plays a role in the defense response of Arabidopsis to herbivore feeding.

In conclusion, we demonstrated that plants are highly flexible in recognizing different attackers and respond by inducing an attacker-specific signal signature and transcript profile. Although one can make predictions based on signal signature and transcript profile whether or not prior attack by one invader will affect the resistance against a subsequent attacker, we have shown that these predictions are not necessarily correct. In particular, specialized attackers might manipulate pathway cross-talk for their own benefit and thereby make the host plant more susceptible to subsequent infection by pathogenic micro-organisms or infestation by herbivorous insects. Hence, this research provided new insights into the complexity of the plant's response to harmful organisms. However, many intriguing questions remain on the continuing arms race between a host and its specialized attackers.