SUMMARY

The survival rate of plants depends on their efficient mechanism to handle the adverse conditions present in the natural environment. In addition to abiotic types of stress, like drought, saline soil, temperature, or high intensity light, plants are under continuous threat of attack by a variety of pathogens. Upon pathogen attack, the plant may respond by activating defense measures through signaling hormones including salicylic acid (SA). SA is typically involved in mediating defense against biotrophic pathogens. The current knowledge of the SA-mediated signaling pathway and the transcriptional regulation of defense responses mediated through this signal molecule is reviewed in **Chapter 1.**

The accumulation of SA in systemic leaves leads to the onset of systemic acquired resistance (SAR). SAR is a broad-spectrum plant defense mechanism that is engaged upon a diversity of plant/pathogen interactions. The enzymatic activity of various PR proteins is well characterized and functionally correlates with resistance against pathogens, although the function of other PR proteins is still not understood. Among these is PR-1, which is generally used as marker for SAR. WRKY proteins belong to a plant-specific class of transcription factors. There are 74 WKRY genes identified in Arabidopsis. In Arabidopsis and other plants many WRKY proteins are involved in responses to stress, especially to biotic stress. WRKY proteins bind to DNA at so-called W-boxes, DNA elements with the sequence TTGAC(T/C), and they may act either as transcriptional activators or as repressors of genes that play roles in the stress response. We are interested in the transcriptional activation of defense during SAR and have used the tobacco and Arabidopsis *PR-1* genes as model genes in our studies.

Previous work by others has indicated the importance of a region in the promoter of the *PR-1* gene for SA-induced expression. This region contains

several potential binding sites for transcription factors that could be involved in the induced expression of *PR-1*.

In **Chapter 2** we have shown that WRKY50 binds to the *PR-1* promoter at two specific positions in close proximity to binding sites of TGA proteins, members of the bZIP class of DNA-binding transcription factors. The two WRKY50 binding sites were highly similar in sequence, but surprisingly, they did not resemble the consensus W-box. To validate the role of the WRKY50 binding sites in the promoter protoplast transactivation assays were performed, which showed that WRKY50 is able to activate *PR-1* gene expression. Combined mutations of these two binding site completely abolished expression of *PR-1*-controlled reporter gene expression in Arabidopsis protoplasts.

Chapter 3 deals with the effects of combinations of WRKY50 and TGA proteins on *PR-1* gene expression. We found that WRKY50 interacts with TGA2 and TGA5 in protein-protein interaction studies in yeast and in planta. Co-expression of AtWRKY50 with TGA2 or TGA5 synergistically enhanced *PR-1* gene expression in protoplast transactivation assays. These findings support the idea that WRKY50 and TGA2 and TGA5 co-operate in the regulation of *PR-1* expression. In addition to the two binding sites for WRKY50, the *PR-1* promoter contains a number of W-boxes that have been shown to be also important for expression. In **Chapter 4** we describe that AtWRKY28 is able to bind to these W-boxes and to activate *PR-1* gene expression in the protoplast transactivation assay.

Chapter 5 describes the effects of overexpression of the WRKY50 and WRKY28 proteins in transgenic plants. Also T-DNA insertion mutants of *WRKY50* were characterized. High constitutive expression of the *WRKY50* gene resulted in higher accumulation of *PR-1* mRNA when the plants were treated with SA, but without SA treatment *PR-1* expression was not enhanced. This indicates that on its own WRKY50 cannot trigger *PR-1* expression, but once

expression is initiated by SA, WRKY50 supports high level expression. WRKY28 had an opposite effect on *PR-1* expression. SA-induced *PR-1* expression in the WRKY28 overexpression plants was lower than in wild type plants. This supports a role for WRKY28 as a repressor of *PR-1* expression. The overexpression of WRKY50 and WRKY28 had no clear-cut effect on the plants' resistance to the biotrophic bacterial pathogen *Pseudomonas syringae* or the necrotrophic fungal pathogen *Botrytis cinerea*, and neither was this the case with WRKY50 T-DNA knockout plants.

The effects of various WRKYs on the metabolome were investigated in **Chapter 6**. In addition to the overexpression lines of WRKY50 and WRKY28 described in the previous chapter, transgenic plants Arabidopsis plants overexpressing WRKY51 and WRKY46 were generated. To investigate the WRKY's influences on metabolite composition, a ^{1}H NMR spectroscopy-based metabolomic approach was applied. Multivariate data analyses, such as principal component analysis, hierarchical cluster analysis and partial least square-discriminant analysis of the NMR data showed that the metabolome of Arabidopsis overexpressing AtWRKY50 differed from wild type Arabidopsis and transgenic Arabidopsis overexpressing the other WRKY genes. The AtWRKY50 overexpression plants contained two- to three-fold more sinapic acid and sinapoyl glucose. This indicates a possible involvement of AtWRKY50 on secondary metabolite production in Arabidopsis, in particular of hydroxycinnamates such as sinapic acid and sinapoyl glucose. As these compounds are components of lignin, this may point to a role of AtWRKY50 in stress-induced lignin modification.